

**OFFICE OF CLINICAL PHARMACOLOGY REVIEW**

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NDA: 022532	Submission Dates: 8/21/2009, 1/11/2010, 2/3/2010, 3/11/2010, 3/22/2010, 4/16/2010, 4/23/2010, 5/7/2010, 5/27/2010, 7/16/2010
Brand Name	Beyaz
Generic Name	Drospirenone, ethinyl estradiol, and levomefolate calcium
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OCP Division	Division of Clinical Pharmacology 3
OND Division	Division of Reproductive and Urologic Products
Sponsor	Bayer
Submission Type, Code	Original, 1S
Related IND	IND 72287
Formulation; Strength(s)	Oral tablet containing either 1) drospirenone 3 mg, ethinyl estradiol 0.02 mg, and levomefolate calcium 0.451 mg or 2) levomefolate calcium 0.451 mg only
Indications	<ul style="list-style-type: none"><li>• Prevention of pregnancy</li><li>• Treatment of symptoms of premenstrual dysphoric disorder (PMDD) in women who choose to use an oral contraceptive as their method of contraception</li><li>• Treatment of acne for women of at least 14 years old</li><li>• Improvement in folate status in women who elect to use an oral contraceptive</li></ul>

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## 1 Executive Summary

Beyaz consists of 24 hormone containing tablets (hereafter referred to as Beyaz hormone) each containing 3 mg of drospirenone (DRSP), 0.02 mg of ethinyl estradiol (EE) stabilized by betadex as a clathrate (molecular inclusion complex) and 0.451 mg of levomefolate calcium (Metafolin), and 4 tablets containing 0.451 mg of Metafolin only (Metafolin mono). Metafolin is a crystalline form of the calcium salt of L-5-methyltetrahydrofolate (L-5-MTHF). The dosage of Beyaz is one hormone-containing tablet daily for 24 consecutive days followed by one Metafolin mono tablet daily for 4 days per treatment cycle.

There are 4 proposed indications for Beyaz:

1. Prevention of pregnancy
2. Treatment of symptoms of premenstrual dysphoric disorder (PMDD)
3. Treatment of moderate acne for women of at least 14 years old
4. Improvement in folate status

The first 3 indications are the same as in approved product Yaz (NDAs 21676, 21873, and 22045), and are supported by a bioequivalence (BE) study (study 309664) comparing the pharmacokinetics (PK) of Beyaz to Yaz with respect to the DRSP and EE components. Yaz is an approved product containing 24 tablets each containing 3 mg of DRSP and 0.02 mg of EE (hereafter referred to as Yaz hormone), and 4 placebo tablets. The difference between Beyaz and Yaz is the addition of 0.451 mg of Metafolin in each Beyaz tablet.

The fourth indication, improvement in folate status, is a new indication that has not been approved for any other drugs. It is supported by 2 pharmacodynamic (PD) studies (studies 310662 and 309763). Study 310662 evaluated increases in red blood cell (RBC) and plasma folate concentrations following daily administration of 0.451 mg Metafolin (from Beyaz) for 24 weeks. Study 309763 also evaluated folate concentrations but women were administered either 1) a combination of a Yasmin® tablet (an approved product consists of 21 tablets each containing 3 mg DRSP and 0.03 mg EE [hereafter referred to as Yasmin hormone], and 7 placebo tablets) and a folic acid 0.4 mg tablet or 2) a combination regimen called Yasmin + Metafolin. Yasmin + Metafolin contains 21 tablets each containing 3 mg DRSP, 0.03 mg EE, and 0.451 mg Metafolin (hereafter referred to as Yasmin + Metafolin hormone) and 7 tablets each containing 0.451 mg Metafolin.

In addition to these studies, the NDA includes a second BE study (study 309662), which provided additional PK information for the Metafolin mono tablet. A summary of literature references related to absorption, distribution, metabolism, and excretion (ADME) properties of Metafolin and drug interactions was also provided in the NDA.

The Clinical Pharmacology review focused mainly on the BE studies and summary of ADME information. The 2 PD studies in support of improving the folate status are reviewed by the Clinical team.

### 1.1 Recommendation

The Division of Clinical Pharmacology 3/Office of Clinical Pharmacology finds NDA 022532 Acceptable from a Clinical Pharmacology perspective, pending agreement on labeling changes.

### 1.2 Phase IV Commitments

None.

### 1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Beyaz differs from the approved Yaz product by the addition of 0.451 mg Metafolin in each tablet (both hormone-containing and non hormone-containing tablets). The acid form of metafolin is L-5-MTHF, the most common form of folate in systemic circulation. The addition of metafolin is to provide folate supplementation. The sponsor is seeking an indication of improving folate status in women on oral contraceptive for the prevention of pregnancy. If approved, this would be the first product to have such indication.

The dose of 0.451 mg Metafolin is equimolar to 0.4 mg folic acid, a dose commonly formulated in vitamin supplements. Metafolin is currently available over the counter as a dietary supplement.

#### **Pharmacokinetics of EE, DRSP and Metafolin:**

Table 1 shows the mean EE and DRSP PK parameter values following a single dose administration of a Beyaz hormone tablet.

**Table 1: Arithmetic mean ( $\pm$ SD) PK parameters following single dose of Beyaz hormone**

Drug substance	Parameter				
	$C_{max}$ (pg/mL)	$T_{max}^*$ (h)	$T_{1/2}$ (h)	AUC (pg*h/ml)	AUC(0-tlast) (pg*h/ml)
EE	44.1 $\pm$ 14.1	1.52 (1 - 4.18)	10.4 $\pm$ 3.70	451 $\pm$ 167	390 $\pm$ 129
DRSP	27.6 $\pm$ 6.97	2 (0.5 – 4.1)	31.0 $\pm$ 9.14	437 $\pm$ 137	400 $\pm$ 125

\* = Median (range)

Following a single dose administration of a Beyaz hormone tablet, serum L-5-MTHF concentration reached a baseline-corrected mean (SD)  $C_{max}$  of 46.5 (14.3) nmol/L. The median  $T_{max}$  was 0.5 hours (range 0.5 – 4 hours). Mean AUC0-tlast was 222 (63) nmol/L\*hour. The apparent  $t_{1/2}$  was 4.68 (0.47) hours. Similar PK parameter values were obtained following a single dose administration of Metafolin mono tablet.

#### **Distribution, metabolism, and excretion properties of Metafolin:**

The sponsor did not conduct any studies with Beyaz to characterize the distribution, metabolism, and excretion properties of Metafolin. However, the sponsor surveyed the literature and the following pertinent information was gathered.

There is extensive first pass hepatic metabolism following oral folate absorption. Some of the folate uptake by hepatocytes is excreted into the bile which can then be reabsorbed via enterohepatic recirculation. L-5-MTHF has low protein binding in serum (~35% protein bound). It is primarily bound to alpha2-macroglobulin and albumin. Folate kinetics is reported to be biphasic with a fast- and a slow-turnover pool. The fast-turnover pool has a half-life of hours (reported range from 9.9 – 31.5 hours) which probably reflects newly absorbed folate. The slow-turnover pool reflects turnover of folate polyglutamate and has a half life of days (reported range from 9.6 to more than 100 days).

The elimination of folates from the body occurs by urinary excretion of intact folates and catabolic products and fecal excretion.

#### **Intrinsic factors:**

Limited data from the literature suggests that renal impairment may increase the exposure of L-5-MTHF. No information is available on the effects of hepatic impairment or other intrinsic factors on the PK of L-5-MTHF.

No information was provided for DRSP and EE. The sponsor relied entirely on approved label for Yaz.

**Extrinsic factors:**

Reports in the literature indicate that several drugs can reduce folate concentrations. The suggested mechanisms include: inhibition of the human dihydrofolate reductase (e.g. methotrexate and sulfasalazine), reduction of folate absorption (e.g. cholestyramine), and unknown mechanisms (e.g. antiepileptics such as carbamazepine, phenytoin, and valproic acid). The effects of these drugs on the bioavailability of Metafolin are not known.

Literature reports indicate that folates may alter the PK or PD of certain antifolate drugs such as phenytoin, methotrexate, and pyrimethamine.

No information was provided for DRSP and EE. The sponsor relied entirely on approved label for Yaz.

**Pharmacogenomics:**

Based on Sponsor's descriptive results, there does not appear to be significant relationship between the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677 C>T variant and folate responses to Yaz or Beyaz. Mean RBC and plasma folate levels at Baseline were comparable for the wild type (CC) and heterozygote (CT) genotype groups and lowest for the variant (TT) genotype group. For the homozygote (variant) TT genotype group of MTHFR polymorphism 677C>T, an increase in plasma and RBC folate was observed in the Beyaz group that was similar to the CC and the CT genotype groups. For homocysteine, Baseline level was slightly higher in the TT (variant) genotype group compared with the other genotype groups. During treatment, homocysteine levels were similar for all genotypes.

**Bioequivalence of DRSP and EE in Beyaz and Yaz:**

The sponsor conducted a single dose BE study to compare the bioavailability of Beyaz hormone tablets (test) to Yaz hormone tablets (reference) under a fasting state. The results showed that the 90% CIs for test/reference ratio for DRSP and EE C<sub>max</sub> and AUC<sub>tlast</sub> were within the 80 – 125% BE limits indicating that the 2 formulations were bioequivalent with respect to DRSP and EE.

**Formulation:**

Both Beyaz hormone and Metafolin mono tablets were formulated as immediate release tablets. The Beyaz hormone and Metafolin mono formulations used in BE study 309664 and PD study 310662 were the same as the proposed to-be-marketed formulation. Throughout the clinical development program for Beyaz, the tablet composition and manufacturing processes were not changed.

**Bioanalytical methods:**

Assays for BE studies:

Plasma DRSP, plasma EE, and serum L-5-MTHF concentrations from BE studies 309664 and 309662 were measured using validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methods. Inspection of the bioanalytical site for study 309664 revealed issues with robustness of the lower limit of quantitation (LLOQ) for DRSP and EE. Other issues specific to the DRSP assay included 1) failure to reject certain runs (due to failed acceptance criteria, chromatographic interference, or unacceptable acceptance practice), and 2) analysis of samples outside of established storage stability range. The Sponsor was requested to raise the LLOQ for DRSP and EE assays and to exclude samples that did not meet acceptance criteria. The Sponsor complied and the revised dataset was considered acceptable.

Folate assay for PD studies:

Total folate in plasma and whole blood concentrations from PD studies 309763 (Yasmin + Metafolin compared to Yasmin and 0.4 mg/day folic acid) and 310662 (Beyaz compared to Yaz) were measured using a microbiological assay with *Lactobacillus casei*. The assay had a calibration range of 25 – 500 ng/ml (56.64 – 1132.76 nmol/L) in blood and 1 – 20 ng/mL (2.27 – 45.31 nmol/L) in plasma. The validation report stated that the assay was not robust. Many plates were rejected due to calibration and/or quality control (QC) samples not meeting acceptance criteria. In some runs the calibration curve flattens at higher concentration and causing high bias for the High QC samples.

QC at LLOQ did not meet the  $\pm 20\%$  criteria for accuracy and precisions. The bias in accuracy at LLOQ was -21.8% in whole blood and -23.3% in plasma. Intra run imprecision at LLOQ was 19.4% in whole blood and 22.7% in plasma. Inter run imprecision at LLOQ was 27.6% in whole blood and 36.3% in plasma. Accuracy and precision criteria were met at other QC levels including QC Low (i.e., 75 ng/mL for whole blood and 3 ng/mL for plasma). Therefore, the assay had an “effective LLOQ” of 75 ng/mL in whole blood and 3 ng/mL in plasma. All QC samples used during bioanalysis of studies 309763 and 310662 were prepared in 5% BSA. Review of additional data from spiked plasma and blood samples by Division of Scientific Investigation (DSI) indicated that there was sufficient accuracy at the respective “effective LLOQ” levels.

#### Metabolite assay:

Concentrations of folic acid, tetrahydrofolate (THF), 5-methyl-THF, 5-formyl-THF, and 5,10-methyl-THF from study 309763 were measured using a semi-quantitative LC/MS assay. The assay was based on acceptance criteria of 50% for accuracy and precision at LLOQ (40% at other QC levels). The calibration range was 0.5 – 150 ng/mL. The QC levels (LLOQ, QC Low, QC Medium, and QC High) were at 0.5, 1.5, 20, and 100 ng/mL for 5-methyl-THF, and 0.5, 1.5, 5, and 10 ng/mL for the remaining analytes.

Available data supported the stability of folic acid and L-5-MTHF; bioanalytical results for these 2 moieties could be reviewed (taking into account the semi-quantitative nature of the assay). Long term and short term stability of 5-FTHF, 5,10-MTHF, and THF were not established. Available data suggests that 5,10-MTHF and THF are not stable following a freeze thaw cycle. Therefore, bioanalytical results for 5-FTHF, 5,10-MTHF, and THF are not considered reliable.

Briefing: An Optional Inter-Division Level Office of Clinical Pharmacology Briefing was held on July 20, 2010 with the following in attendance: Doanh Tran, Christian Grimstein, Li Zhang, Chinmay Shukla, Hae Young Ahn, Chongwoo Yu, Liang Zhao, Sayed Al Habet, LaiMing Lee, Daniel Davis, Lisa Soule, Myong Jin Kim, and E. Dennis Bashaw.

## 2 Question Based Review

### 2.1 General Attributes

#### 2.1.1 What is Beyaz? What are the proposed indications for Beyaz?

Beyaz consists of 24 tablets each containing 3 mg of DRSP, 0.02 mg of EE stabilized by betadex as a clathrate (molecular inclusion complex) and 0.451 mg of Metafolin (Beyaz hormone), and 4 tablets containing 0.451 mg of Metafolin only. Both Beyaz hormone and Metafolin mono tablets are formulated as immediate release tablets. The dosage of Beyaz is one hormone-containing tablet daily for 24 consecutive days followed by one Metafolin mono tablet daily for 4 days per treatment cycle.

The proposed indications for this product are as follows:

1. Prevention of pregnancy
2. Treatment of symptoms of premenstrual dysphoric disorder (PMDD)
3. Treatment of moderate acne for women of at least 14 years old
4. Improvement in folate status

The first 3 indications are the same as in approved product Yaz (NDAs 21676, 21873, and 22045), and are supported by a BE study comparing the PK of Beyaz to Yaz with respect to the DRSP and EE components.

The fourth indication is supported by 2 PD studies showing increased RBC and plasma folate due to administration of Metafolin. Metafolin is a crystalline form of the calcium salt of L-5-MTHF. L-5-MTHF is the predominant form of dietary folate and is the only species normally found in the circulation.

#### 2.1.2 What Clinical Pharmacology data were provided in this NDA?

The NDA is supported mainly by BE study 309664 and 2 PD studies (studies 309763 and 310662). The endpoints for the PD studies were plasma folate, red blood cell folate, and plasma homocysteine concentrations. A second BE study (study 309662) provided additional PK information for the Metafolin mono tablet. Additional supporting data included a PK/PD population analysis using data from the above 4 studies, a relative bioavailability analysis of a literature report comparing L-5-MTHF and folic acid [Langenohl et al. 2003], and literature references related to ADME properties, drug interaction, and PD of levomefolate calcium.

*Review note: The EE and DRSP data from BE study 309662 comparing Yasmin + Metafolin, Yasmin, and Metafolin mono was not considered in this review. Study 309662 was not essential for evaluation of safety and efficacy of Beyaz. Furthermore, the reported EE and DRSP results for study 309662 may need to be substantially revised pending a DSI inspection of the bioanalytical site. Only L-5-MTHF data from this study were considered as supportive data.*

### 2.2 General Clinical Pharmacology

Beyaz contains Metafolin and the same dose of active ingredients in the approved product Yaz, namely DRSP and EE. The safety and efficacy of DRSP and EE in Beyaz is supported solely by the BE study bridging to Yaz. No additional clinical pharmacology information with respect to DRSP and EE was submitted in this NDA. Therefore, the Clinical Pharmacology review is mainly focused on the new active ingredient Metafolin, in addition to the BE assessment for DRSP and EE. For additional clinical

pharmacology information on DRSP and EE, please refer to product label for Yaz and clinical pharmacology reviews of Yaz NDAs (NDAs 021676, 021873, and 022045).

2.2.1 What are the pharmacokinetic properties of the Beyaz hormone tablet? Are Beyaz hormone tablets BE to Yaz hormone tablets with respect to DRSP and EE?

Bioavailability of Beyaz hormone tablets was evaluated in single dose, crossover BE study 309664. This study evaluated plasma DRSP and EE concentrations and serum L-5-MTHF concentrations following a single oral dose of Beyaz hormone or Metafolin mono tablet in 42 healthy young females. This study also provided BE bridging information for EE and DRSP between Yaz hormone and Beyaz hormone tablets. Each Yaz hormone tablet contains 3 mg DRSP and 0.02 mg EE.

**EE:**

For Beyaz hormone tablets, the mean ( $\pm$ SD) EE  $C_{max}$  was  $44.1 \pm 14.1$  pg/mL and was reached 1.52 hours (range: 1 to 4.2 hr) after administration. Mean values for AUC(0–tlast) was  $390 \pm 129$  pg\*h/mL. The AUC and  $t_{1/2}$  were  $451 \pm 167$  pg\*h/mL and  $10.4 \pm 3.70$  hr respectively (table 2). The AUC and  $t_{1/2}$  could be determined only for 8 volunteers, due to insufficient time range for half-life calculation of less than two half-lives and/or an extrapolated part of the AUC greater than 20% or due to the exclusion of subjects.

The mean PK profiles for Beyaz hormone and Yaz hormone tablets were similar (figure 1). The results of BE analysis showed that the 90% CI of Beyaz/Yaz ratio for EE  $C_{max}$  and AUC were within the 80 – 125% limits, indicating that Beyaz and Yaz were BE with respect to EE.

**Table 2: Arithmetic mean ( $\pm$  SD) pharmacokinetic parameters of EE**

Treatment	Parameter				
	$C_{max}$ (pg/mL)	$T_{max}^*$ (h)	$T_{1/2}$ (h)	AUC (pg*h/ml)	AUC(0-tlast) (pg*h/ml)
Beyaz (N=39)	$44.1 \pm 14.1$	1.52 (1 - 4.18)	$10.4 \pm 3.70$ (N=8)	$451 \pm 167$ (N=8)	$390 \pm 129$
Yaz (N=39)	$41.6 \pm 13.1$	1.5 (0.5 - 4)	$9.71 \pm 3.76$ (N=14)	$452 \pm 155$ (N=14)	$379 \pm 136$
* = Median (range)					

**Figure 1: Mean plasma concentrations of EE (ZK4944)**

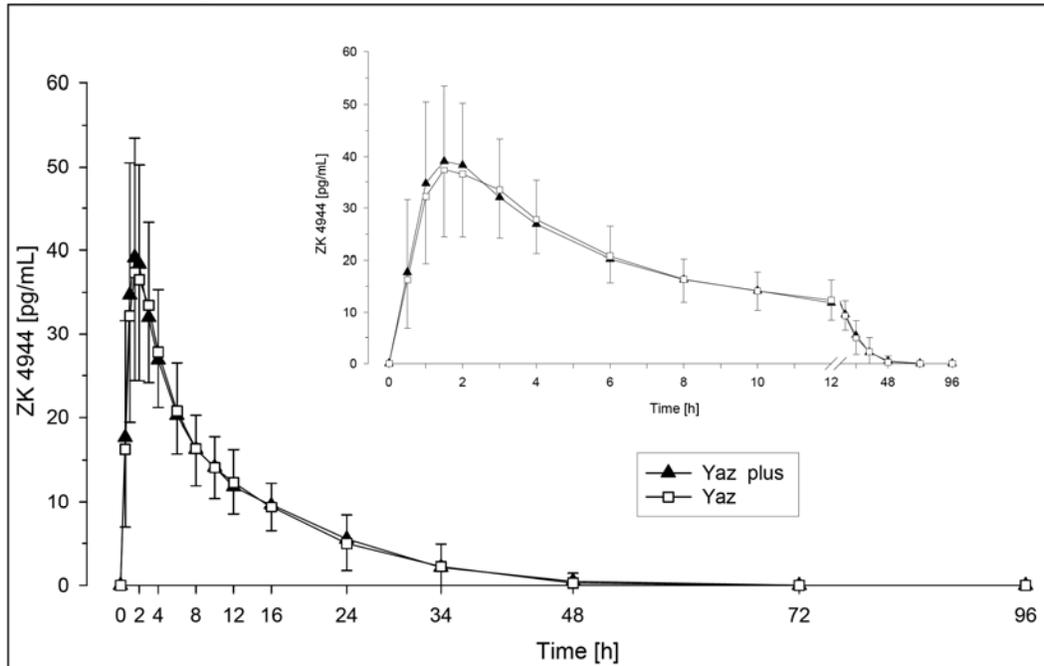


Figure note: Yaz plus = Beyaz

**Table 3: Bioequivalence assessment for EE**

EE	N	Yaz (reference)	Beyaz (test)	Test/reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [pg*h/ml]	38	379.0	388.7	103.5	(99.2, 108.0)
Cmax [pg/mL]	38	41.7	44.1	106.0	(98.9, 113.7)

Note that for the geometric means and bioequivalence assessment of AUC(0-tlast), only data from 38 subjects was used (data for both reference and test available). RNR 39 and RNR 40 had missing values.

**DRSP:**

For Beyaz hormone tablets, the mean ( $\pm$ SD) DRSP  $C_{max}$  in Beyaz was  $27.6 \pm 6.97$  ng/mL and was reached 2 hours (range: 0.5 to 4.1 hr) after administration. Mean values for AUC(0-tlast) was  $400 \pm 125$  ng\*h/mL. The AUC and  $t_{1/2}$  were  $437 \pm 137$  ng\*h/mL and  $31 \pm 9.14$  hr, respectively (table 4).

The mean DRSP PK profiles for Beyaz hormone and Yaz hormone tablets were similar (figure 2). The results of BE analysis showed that the 90% CI of Beyaz/Yaz ratio for DRSP  $C_{max}$  and AUC were within the 80 – 125% limits, indicating that Beyaz and Yaz were BE with respect to DRSP.

**Table 4: Arithmetic mean ( $\pm$  SD) pharmacokinetic parameters of DRSP**

Treatment	Parameter				
	$C_{max}$ (pg/mL)	$T_{max}^*$ (h)	$T_{1/2}$ (h)	AUC (pg*h/ml)	AUC(0-tlast) (pg*h/ml)
Beyaz (N=36)	$27.6 \pm 6.97$	2 (0.5 – 4.1)	$31.0 \pm 9.14$ (N=33)	$437 \pm 137$ (N=33)	$400 \pm 125$
Yaz (N=35)	$26.2 \pm 6.44$	2 (0.5 – 4)	$31.3 \pm 8.27$ (N=34)	$437 \pm 115$ (N=34)	$400 \pm 110$

\* = Median (range)

**Figure 2: Mean plasma concentrations of DRSP (ZK30595)**

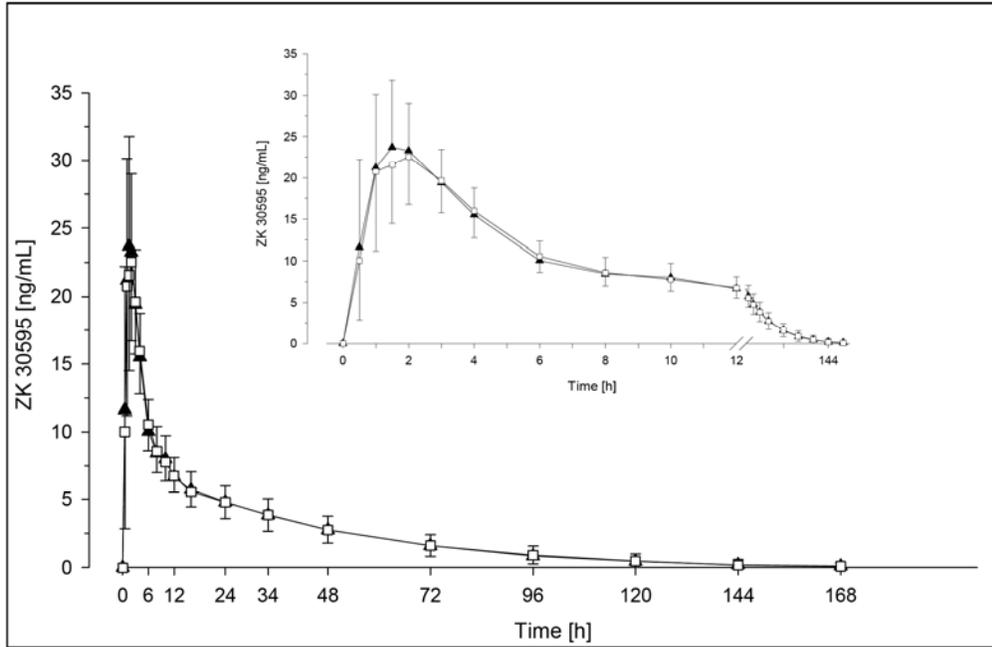


Figure note: open squares = Yaz; closed triangles = Beyaz

**Table 5: Bioequivalence assessment for DRSP**

DRSP	N	Yaz (reference)	Beyaz (test)	Test/reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [pg*h/mL]	34/35	396.8	397.9	100.5	(97.3, 103.8)
Cmax [pg/mL]	34/35	26.3	27.7	105.2	(97.3, 113.8)

Note that for bioequivalence assessment, only data from 33 subjects was used (data for both reference and test available). For calculation of the geometric mean for reference treatment 34 subjects contributed data (RNR 28 had valid data), whereas for test treatment 35 subjects contributed data (RNR 20 and RNR 102 had valid data).

**L-5-MTHF:**

Table 6 shows the PK parameters for serum L-5-MTHF following single dose administration of Beyaz hormone and Metofolin mono tablets. Beyaz hormone showed a median  $T_{max}$  of 0.5 hours for L-5-MTHF. The apparent (baseline corrected)  $t_{1/2}$  was 4.7 hours. Figures 3 and 4 show the mean serum L-5-MTHF PK profiles for baseline-uncorrected and baseline-corrected, respectively. The figures include mean profiles following treatment with Yaz, which represents the profile without administration of metafolin.

Baseline correction was performed by subtracting the value of predose measurement (at -30 minutes) from each observed concentration. Individual L-5-MTHF concentration time profiles in Yaz treated period (i.e., no Metafolin) showed a flat pattern, indicating that a single predose sample was adequate to capture baseline L-5-MTHF concentration.

**Table 6: Arithmetic mean ( $\pm$ SD) L-5-MTHF PK parameters (Study 309664)**

Baseline method	Treatment	C <sub>max</sub> (nmol/L)	AUC <sub>0-tlast</sub> (nmol/L*h)	T <sub>max</sub> * (h)	T <sub>1/2</sub> (h)
Baseline - uncorrected	Beyaz (n=40)	60.4 $\pm$ 18.0	391 $\pm$ 137	0.5 (0** - 4)	NC
	Metafolin mono (n=40)	61.2 $\pm$ 21.8	391 $\pm$ 137	0.5 (0.5 - 2)	NC
Baseline - corrected	Beyaz (n=39)	46.5 $\pm$ 14.3	222 $\pm$ 63	0.5 (0.5 - 4)	4.68 $\pm$ 0.474
	Metafolin mono (n=40)	47.5 $\pm$ 18.9	226 $\pm$ 68	0.5 (0.5 - 2)	4.54 $\pm$ 0.504

\* T<sub>max</sub> is shown as median and range.  
 \*\* One subject (RNR 004) had T<sub>max</sub> at predose time point  
 NC Not calculated

**Figure 3: Mean serum concentration ( $\pm$ SD) of baseline-uncorrected L-5-MTHF (ZK270898)**

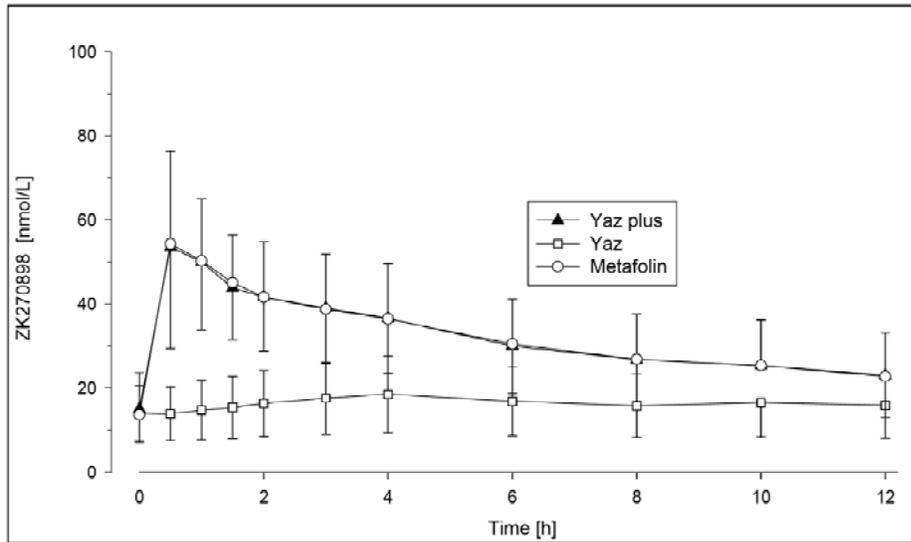


Figure note: Yaz plus = Beyaz; Yaz does not contain Metafolin and L-5-MTHF profile for Yaz represents endogenous concentrations.

**Figure 4: Mean serum concentration ( $\pm$ SD) of baseline-corrected L-5-MTHF (ZK270898)**

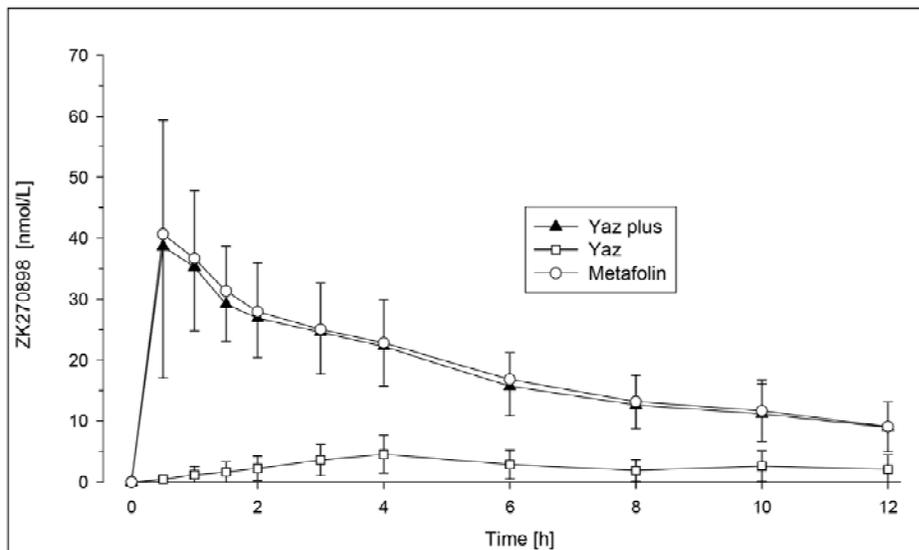


Figure note: Yaz plus = Beyaz; Yaz does not contain Metafolin and L-5-MTHF profile for Yaz represents endogenous concentrations.

### 2.2.2 What are the pharmacokinetic properties of Metafolin mono tablet?

As discussed in section 2.2.1, BE study 309664 included administration of Metafolin mono tablet in one treatment period. The PK parameters and profile of L-5-MTHF following a single dose administration of Metafolin mono tablet is shown in Table 6 and figure 3, respectively. The results showed similar PK properties for Metafolin mono tablet and Beyaz hormone tablet. The Sponsor performed BE analyses comparing baseline-corrected and baseline-uncorrected L-5-MTHF between Beyaz hormone and Metafolin mono tablets. These BE comparisons were not required from a regulatory perspective. Nevertheless, the results showed that the 90% confidence intervals for the ratios of geometric means for  $C_{max}$  and AUC were within the 80 – 125% range, indicating the both the Beyaz hormone and Metafolin mono tablets provided the same L-5-MTHF bioavailability.

The sponsor asserted that these results also indicate that there are no metabolic interaction between DRSP/EE and L-5-MTHF. Since the formulation for Beyaz hormone and Metafolin only tablets are similar (see section 2.5.2), this reviewer agrees that there likely was no metabolic interaction present following this single dose administration. However, potential confounding effects of the different formulations could not be completely ruled out. It should also be noted that this was a single dose study and may not capture the maximum interaction potential between DRSP/EE and L-5-MTHF. The product label for Yaz indicates that upon multiple dose administration, serum  $C_{max}$  and AUC accumulated by about 2 – 3 fold for DRSP and about 1.5 – 2 fold for EE.

**Table 7: Bioequivalence assessment for L-5-MTHF (baseline-corrected, PPS)**

L-5-methyl-THF	N	Metafolin (reference)	Beyaz (test)	Relative bioavailability (test / reference) in %	Two-sided 90% confidence interval in %
AUC(0–tlast) [nmol*h/l]	40 / 39	225.76	222.80	98.16	( 93.95 , 102.57 )
Cmax [nmol/l]	40 / 39	47.46	46.50	99.88	( 91.24 , 109.34 )

Note: ANOVA was based on 39 complete data sets

**Table 8: Bioequivalence assessment for L-5-MTHF (baseline-uncorrected, PPS)**

L-5-methyl-THF	N	Metafolin (reference)	Beyaz (test)	Relative bioavailability (test / reference) in %	Two-sided 90% confidence interval in %
AUC(0–tlast) [nmol*h/l]	40	390.99	391.07	99.88	( 95.38 , 104.58 )
Cmax [nmol/l]	40	61.19	60.40	100.28	( 93.15, 107.95 )

### 2.2.3 What is the relative bioavailability of Metafolin and folic acid?

It is difficult to provide a conclusive answer to this question because there are many formulations of folic acid available as over-the-counter and they may potentially have different absolute bioavailability due to differences in formulation performance. Nevertheless, available data suggest that administration of an equimolar dose of Metafolin would yield similar or higher folate exposure compared to administration of folic acid. PD study (study 309763) of Yasmin + Metafolin (combination tablet containing 0.451 mg Metafolin), and Yasmin + a 0.4 mg folic acid tablet provided a relative comparison of the degree of folate supplementation from the 2 specific formulations evaluated. The results indicated that the steady state plasma and red blood cell folate concentrations were similar in women taking Yasmin + Metafolin or Yasmin + 0.4 mg folic acid. Additionally, a study in the literature [Prinz-Hangenohl et al., 2003] evaluated the relative bioavailability of single doses of 0.416 mg L-5-MTHF (906 nmol, equivalent to 0.451 mg Metafolin) or 0.400 mg folic acid, with or without folic acid preload (1 mg/day for 10 days), in 21 healthy female volunteers. The authors reported that the bioavailability of L-5-MTHF (baseline corrected AUC) was equivalent (with folic acid preload) or higher (without folic acid preload [AUC ratio 156%]) than an equimolar dose of folic acid (i.e., 0.400 mg). It should be noted that the folic acid formulations used in these studies may have different bioavailability compared to those used in studies relating to prevention of neural tube defects (NTD).

### 2.2.4 Does administration of Beyaz lead to increase in plasma and RBC folate concentration?

The effect of 24 weeks administration of 0.451 mg levomefolate calcium on plasma folate, RBC folate, and plasma homocysteine concentrations were compared to no folate administration in study 310662 (Beyaz vs. Yaz) and to 0.4 mg/day folic acid in study 309763 (Yasmin + Metafolin vs. Yasmin and 0.4 mg/day folic acid). The details of these studies are reviewed and discussed in the Clinical review by Dr. Daniel Davis, Medical Officer.

The results (as reported by sponsor) indicated that daily administration of 0.451 mg Metafolin increased plasma and RBC folate concentrations. The geometric mean plasma and RBC folate concentrations after once daily administration of Beyaz for 24 weeks were 57.5 nmol/L and 1346 nmol/L, respectively. The geometric mean plasma and RBC folate concentrations after once daily administration of Yasmin + Metafolin for 24 weeks were 47.8 nmol/L and 1324 nmol/L, respectively.

The increase in plasma and RBC folate concentrations were observed regardless of whether women were from area with or without a food folate fortification program. However, women from areas with a food folate fortification program had higher baseline folate and a smaller net increase due to Metafolin administration. For example, larger increase was observed in study 309763 (mean increase of 30.5 nmol/L [95% CI 27.4, 33.9 nmol/L] at 24 weeks from a baseline of 15.0 [95% CI 13.6, 16.4]) which was conducted in Germany, where there is no food fortification program. Study 310662, which was conducted

in the US where there is a food fortification program, showed a mean baseline plasma folate concentration of 41.3 nmol/L [95% CI 38.8, 43.9 nmol/L] and a smaller net increase of 16.0 nmol/L [95% CI 13.8, 18.6 nmol/L] at 24 weeks. Similar observations were made for RBC folate concentrations.

The sponsor also provided a population PK/PD report evaluating the kinetics of folate in plasma and RBCs. The PK/PD modeling did not provide additional insights beyond what was already apparent. Due to long runtime, the sponsor did not evaluate effects of covariates. Differences in assays used for PK and PD studies also presented additional challenge in interpreting the modeling results. Therefore, PK/PD results were not reviewed in details.

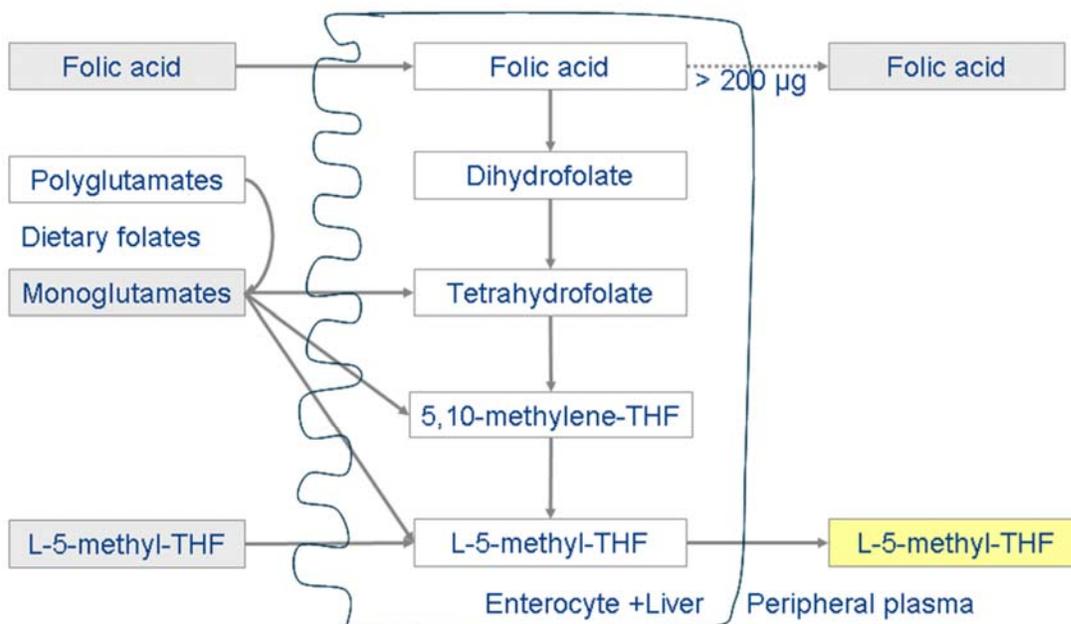
### 2.2.5 What are the ADME properties of Metafolin?

Other than BE study 309664, which provided information on the absorption of L-5-MTHF in the Beyaz formulation, the sponsor did not conduct any other studies relating to ADME properties of L-5-MTHF from Beyaz. The sponsor surveyed the literature and provided a summary of ADME information on folate in general, which in many cases are applicable to L-5-MTHF. This reviewer reviewed the sponsor's summary and associated references when needed. Several review articles/book chapters were relied upon for general information on folate. The following is a summary of relevant ADME properties.

#### Absorption:

Absorption of folates is a saturable, pH-dependent process that occurs throughout the length of the small intestine, but absorption is most efficient in the proximal small intestine [Steinberg 1984]. Polyglutamates from food are deconjugated by glutamate carboxypeptidase II into monoglutamates prior to absorption in the small intestine. However, metabolism is not required for transport across the intestine. Following administration of folic acid, particularly at high doses, unmetabolized folic acid could be detected in the systemic circulation. L-5-MTHF is absorbed unchanged. 5-MTHF is the most common form of folates circulated in humans.

**Figure 5: schematic of folates absorption**



After oral administration of Metafolin (from Beyaz tablets), absorption was rapid with the median  $T_{max}$  for L-5-MTHF of 0.5 hour (range 0.5 to 1.5 hour). Mean serum L-5-MTHF  $C_{max}$  of about 50 nmol/L above baseline was observed following single administration of 0.451 mg Metafolin (based on mean serum L-5-MTHF  $C_{max}$  data from studies 309662 and 309664 as follows: Yasmin + Metafolin:  $53.9 \pm 15.4$  nmol/L, Metafolin mono from study 309662:  $50.7 \pm 13.8$  nmol/L, Beyaz:  $46.5 \pm 14.3$  nmol/L, Metafolin mono from study 309664:  $47.5 \pm 18.9$  nmol/L).

**Distribution:**

There is extensive first pass hepatic metabolism following oral folate absorption [Rogers et al. 1997]. Some of the folate up take by hepatocytes is excreted into the bile which can then be reabsorbed via enterohepatic recirculation.

L-5-MTHF has low protein binding in serum (~35% protein bound based on measurement of folic acid activity in donor serum [Markkanen et al. 1972]). It is primarily bound to alpha2-macroglobulin and albumin.

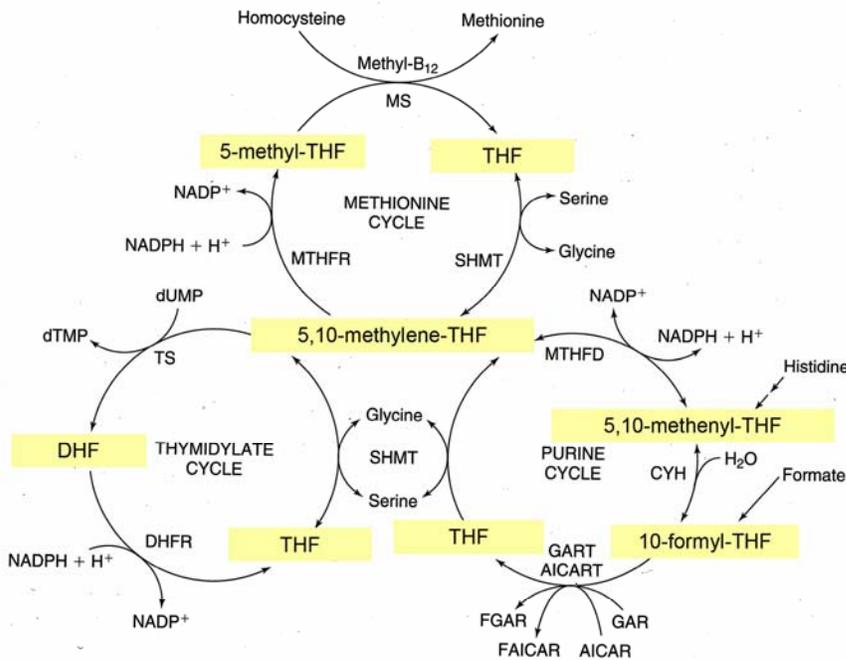
Folate kinetics is reported to be biphasic with a fast- and a slow-turnover pool. The fast-turnover pool has a half-life of hours (reported range from 9.9 – 31.5 hours [von der Prten et al. 1992]) which probably reflects newly absorbed folate. This is in line with the apparent terminal half-life of approximately 4 - 5 hours after single oral administration of 0.451 mg Metafolin. The slow-turnover pool reflects turnover of folate polyglutamate and has a half life of days (reported range from 9.6 to 19 days [von der Porten et al. 1992]) to more than 100 days [Krumdieck et al. 1978]).

L-5-MTHF is the predominant folate in the circulation and therefore likely the folate form normally transported into peripheral tissues to be used for cellular folate metabolism. There are three physiological mechanisms for the transport and the uptake of folates by various cell types: two carrier-mediated, active transport mechanisms (the reduced folate carrier and the folate receptor), and passive diffusion [Sirotnak and Tolner 1999, Henderson et al. 1995].

**Metabolism:**

Accumulation of intracellular folates requires the conversion of monoglutamates into polyglutamate forms by the enzyme folylpolyglutamate synthetase (FPGS). L-5-MTHF is a poor substrate for FPGS [Cichowicz and Shane 1987] suggesting that the incorporation of L-5-MTHF into the cellular folate metabolism is preceded by the conversion to THF via the methionine synthase reaction before effective polyglutamylation and tissue retention is achieved. Folate coenzymes are involved in 3 major interrelated metabolic cycles in the cytosol of cells.

**Figure 6: Folate mediated metabolism (adapted from Shane 2006 by Sponsor)**



Abbreviations: AICAR, aminimidazole carboxamide ribonucleotide; AICART, AICAR formyltransferase; B12, vitamin B12 (cobalamin); CYH, methenyltetrahydrofolate cyclohydrolase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; FAICAR, formyl-aminoimidazole carboxamide ribonucleotide; FGAR, formyl-glycinamide ribonucleotide; GAR, glycinamide ribonucleotide; GART, GAR formyltransferase; MS, methionine synthase; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylenetetrahydrofolate reductase; SHMT, serine hydroxymethyltransferase; THF, L-tetrahydrofolate; TS, thymidylate synthase

### Excretion:

The elimination of folates from the body occurs by urinary excretion of intact folates and catabolic products and fecal excretion. The body folates pools exhibit biphasic kinetics with a rapid and a slow turnover pool. A rapid decline in urinary and fecal radioactivity with a half-life of hours (reported range 9.9 – 31.5 hours) is followed by a long decline with a reported half-life ranging from 9.6 to more than 100 days [von der Porten et al. 1992, Krumdieck et al. 1978]. Buchholz et al. (1999) reported that urinary and fecal excretion data from a male adult suggested that it would take 360 days to completely eliminate an oral dose of 35 µg [14C]-labelled folic acid by linear extrapolation. If exponential extrapolation was done, the elimination  $t_{1/2}$  was estimated to be about 300 days.

### 2.3 Intrinsic Factors

#### 2.3.1 Were the effects of renal impairment or hepatic impairment on the pharmacokinetic of L-5-MTHF evaluated?

No specific studies were conducted to evaluate the effect of hepatic or renal impairments on L-5-MTHF. However, studies for L-5-MTHF in patients with renal or hepatic impairment are not necessary since the use of Beyaz is not recommended in these patients due to existing concerns. The current Yaz label has a warning that “Yaz should not be used in patients with conditions that predispose to hyperkalemia (i.e. renal insufficiency, hepatic dysfunction and adrenal insufficiency).” Additionally, patient with moderate

hepatic impairment had mean DRSP exposure that is approximately 3 times higher than the exposure in women with normal liver function.

Limited data from the literature suggested that patients with renal impairment may have higher exposure following exogenous administration of L-5-MTHF. One literature reported a high dose of L-5-MTHF (17 mg once daily for 12 weeks) was administered to subjects with end stage renal disease [Bostom et al. 2000]. The authors reported that renal impairment resulted in significant elevation in folate concentration. After 12 weeks of daily folate intake, plasma folate concentrations increased by a mean of 838 nmol/L. There was no increase in adverse events reported due to the elevated folate concentration.

### 2.3.2 Are genetic 5,10-methylenetetrahydrofolate reductase (MTHFR) variants associated with differential effects of Beyaz on folate biomarkers?

Based on Sponsor descriptive results, there does not appear to be significant relationship between the MTHFR 677 C>T variant and folate responses to Yaz or Beyaz. Mean RBC and plasma folate levels at Baseline were comparable for the wild type (CC) and heterozygote (CT) genotype groups and lowest for the variant (TT) genotype group. For the homozygote (variant) TT genotype group of MTHFR polymorphism 677C>T, an increase in plasma and RBC folate was observed in the Beyaz group that was similar to the CC and the CT genotype groups. For homocysteine, Baseline level was slightly higher in the TT (variant) genotype group compared with the other genotype groups. During treatment, homocysteine levels were similar for all genotypes. Please refer to Appendix 4.2 Pharmacogenomics Review for more details.

## 2.4 Extrinsic Factors

### 2.4.1 What is the effect of food on the bioavailability of Beyaz tablets?

The effect of food on bioavailability of Beyaz tablets was not evaluated. The label for Yaz states that DRSP and EE C<sub>max</sub> decreased by about 40% due to a high fat meal while DRSP AUC was unchanged. EE AUC decreased by about 20% due to food. The Yaz label states that it can be taken without regards to meals.

There were no data submitted on the effect of food on bioavailability of Metafolin for either Beyaz or Metafolin mono tablets. The sponsor proposed that Beyaz can be taken without regards to meals. Since the Yaz approved product is an immediate release formulation and its label states that Yaz can be taken without regards to meals, there is no need to conduct a fed BE study or a food effect study of the new formulation with respect to assessing the bioavailability of DRSP and EE. The PD study evaluating the total folate exposure administered Beyaz without regards to meals. Therefore, this reviewer concurs that Beyaz could be administered without regards to meals. However, since the effect of food intake on Metafolin bioavailability was not evaluated, the label should be edited to reflect this fact.

#### 2.4.2 What are the effects of L-5-MTHF on the pharmacokinetics of other drugs?

The Sponsor did not conduct any drug interaction study with metafolin. The sponsor conducted a review of the literature. The referenced literature is consistent with the sponsor's conclusion that folates may modify the PK or PD of certain antifolate drugs, e.g. antiepileptics (such as phenytoin), methotrexate, pyrimethamine and may result in decreased pharmacological effect of the antifolate drug. Specific recommendations on dosage adjustments could not be made.

#### 2.4.3 What are the effects of other drugs on the concentration of plasma and RBC folate concentrations?

The Sponsor did not conduct any drug interaction study with metafolin. The sponsor submitted a survey of the literature and indicated that "several drugs have been reported to reduce folate levels and decrease the efficacy of folates by inhibition of the human dihydrofolate reductase (e.g. methotrexate, trimethoprim, sulfasalazine, and triamterene) or by reducing folate absorption (e.g. cholestyramine), or via unknown mechanisms (e.g. antiepileptics such as carbamazepine, phenytoin, and valproic acid)."

Dihydrofolate reductase: The referenced literature supports the effects of methotrexate and sulfasalazine on reducing folate concentration. However, it suggests that trimethoprim and triamterene do not alter serum or RBC folate concentrations.

Cholestyramine: Cholestyramine is a quaternary ammonium salt of a high molecular weight copolymer. The substance is hydrophobic and insoluble in water. In the small intestine, the chloride ions of cholestyramine exchange with bile acids, thereby interrupting bile acid enterohepatic circulation. In addition to binding bile acids, cholestyramine may also bind acidic and neutral drugs and reduce or delay their absorption. Reduced folate concentrations have been reported in patient chronically treated with cholestyramine [Cayen 1985].

Antiepileptic drugs: The sponsor referenced literature reports indicating that antiepileptic drugs like phenytoin, carbamazepine, barbiturates, primidone and valproic acid may induce folate deficiency. There are various suggested mechanisms including induction of liver enzymes [Maxwell et al. 1972], impairment of folate absorption [Ibbotson et al. 1967], competitive interaction between folate co-enzymes and drugs [Girdwood and Lenham 1956], and increased demand for folate as a cofactor in the hydroxylation of the anticonvulsant [Jensen and Olesen 1970].

It should be noted that the above observations are for total folate concentrations in the body. Since metafolin administration is expected to contribute to the body's folate pool, it is likely that these observations would hold in the presence of metafolin administration. However, the magnitude of effects on bioavailability of metafolin is not known.

### 2.5 General Biopharmaceutics

#### 2.5.1 Were the formulations used in the 2 Beyaz clinical studies the same as the proposed to-be-marketed formulation?

Yes. Both clinical studies that administered Beyaz (BE study 309664 and PD study 310662) were performed with the proposed to-be-marketed product. Throughout the whole clinical development the tablet composition and manufacturing processes were not changed.

Yaz product used in the BE study 309664 was the approved product. Yaz tablets used in PD study 310662 were in the color of Beyaz. Yasmin tablets used in PD study 309763 were in the color of the new

combination Yasmin + Metafolin tablets. Release dissolution testing results for Yaz and Yasmin formulations used in the PD studies were provided, which showed that they met the release dissolution specification.

Please note that BE of Beyaz and Yaz with respect to EE and DRSP was discussed under section 2.2.1.

### 2.5.2 What is the formulation composition of Beyaz hormone tablet and Metafolin only tablet?

Tables 9 and 10 show the formulation composition of Beyaz hormone tablets and Metafolin mono tablets, respectively.

**Table 9: Composition of Beyaz hormone tablet**

Composition	Reference to standard	Function	Amount [mg]
<b>Drug substance</b>			
Drospirenone micronized	specification	drug substance	3.000
Ethinylestradiol betadex clathrate micronized <sup>a</sup>	specification	drug substance	0.020
Levonorgestrel calcium micronized	specification	drug substance	0.451
<b>Excipients</b>			
Lactose monohydrate	Ph. Eur., USP/NF, Ph. Jap.	(b) (4)	(b) (4)
Cellulose microcrystalline	Ph. Eur., USP/NF		
Croscarmellose sodium	Ph. Eur., USP/NF, Ph. Jap.		
Hydroxypropylcellulose (b) (4)	Ph. Eur., NF		
Magnesium stearate	Ph. Eur., USP/NF, Ph. Jap.		
<b>Weight (uncoated tablet)</b>			
<b>Film-coating</b>			
(b) (4)	specification	(b) (4)	(b) (4)
or alternatively			
Hypromellose (b) (4)	Ph. Eur., USP/NF, Ph. Jap.		
(b) (4)	Ph. Eur., USP/NF		
Talc	Ph. Eur., USP/NF, Ph. Jap.		
Titanium dioxide	Ph. Eur., USP/NF, Ph. Jap. Directive 95/45/EC		
Ferric oxide red	USP/NF, JPE Directive 95/45/EC		
<b>Weight (film-coating)</b>			
<b>Weight (coated tablet)</b>			<b>82.0000</b>
a calculated as ethinylestradiol			
(b) (4)			

**Table 10: Composition of Metafolin only tablet**

Composition	Reference to standard	Function	Amount [mg]
<b>Drug substance</b>			
Levomefolate calcium micronized	specification	drug substance	0.451
<b>Excipients</b>			
Lactose monohydrate	Ph. Eur., USP/NF, Ph. Jap.		(b) (4)
Cellulose microcrystalline	Ph. Eur., USP/NF		
Croscarmellose sodium	Ph. Eur., USP/NF, Ph. Jap.		
Hydroxypropylcellulose (b) (4)	Ph. Eur., NF		
Magnesium stearate	Ph. Eur., USP/NF, Ph. Jap.		
<b>Weight (uncoated tablet)</b>			
<b>Film-coating</b>			
Lacquer light orange <sup>a</sup>	specification		(b) (4)
or alternatively			
Hypromellose (b) (4)	Ph. Eur., USP/NF, Ph. Jap.		
(b) (4)	Ph. Eur., USP/NF		
Talc	Ph. Eur., USP/NF, Ph. Jap.		
Titanium dioxide	Ph. Eur., USP/NF, Ph. Jap.		
Ferric oxide red	Directive 95/45/EC USP/NF, JPE		
Ferric oxide yellow	Directive 95/45/EC USP/NF, JPE		
	Directive 95/45/EC		
<b>Weight (film-coating)</b>			
<b>Weight (coated tablet)</b>			<b>82.0000</b>
			(b) (4)

## 2.6 Analytical Section

2.6.1 What bioanalytical methods were used to assess concentrations of DRSP, EE, and MTHF in bioequivalence studies 309664 (Beyaz vs. Yaz) and 309662 (Yasmin + Metafolin vs. Yasmin)? Were these methods adequately validated?

Plasma DRSP and EE, and serum L-5-MTHF concentrations from BE studies 309664 and 309662 were measured using LC/MS/MS methods. Review of the method validation reports indicated that the methods met the FDA recommended validation criteria. However, there were storage stability (samples stored for longer than proven stability period prior to analysis) and assay performance issues identified. Following corrective action by the Sponsor, the assay was considered acceptable for analysis of BE study 309664.

A summary of the issues and the corrective actions are presented below.

### Storage stability issues:

Review of the bioanalytical reports indicated a potential issue regarding sample stability for both studies 309664 and 309662 was identified. Requests for clarification were sent to sponsor on 1/25/2010 and 3/2/2010 for studies 309664 and 309662, respectively. The following section summarizes the issues and the outcomes for issues related to study 309664. Sponsor's response related to storage stability of EE and DRSP samples for study 309662 will be addressed in Clinical Pharmacology review of NDA 022574.

1. The MTHF bioanalytical report for study 309664 stated that the maximum duration of sample storage (from first collection date to last analysis) was 335 days. It further stated that stability of MTHF in human serum at -20 °C and -80 °C was demonstrated for 286 days and that stability study will be

extended to cover the study sample storage period. Provide data supporting long term storage stability of MTHF in human serum for at least 335 days. Alternatively, provide evidence that each sample from this study was analyzed for MTHF within 286 days of sample collection.

- Sponsor confirmed that each sample in the final data set from study 309664 was analyzed for MTHF within 286 days of sample collection.
2. The DRSP bioanalytical report for study 309664 states that storage stability for DRSP in human plasma was demonstrated for 200 days. The report indicated that all reported values were based on sample stored for  $\leq 200$  days at (b)(4)1 (except for one sample from subject 8 that were analyzed on 25 October 2007, which was stored for 205 days at (b)(4)). It is not clear if samples were analyzed within 200 days from the day of sample collection (i.e., the total time stored at (b)(4) and time stored elsewhere before being shipped to (b)(4)). It appears that the first sample collected from study 309664 was on 8 January 2007 and the last analytical run for DRSP analysis was performed on 25 October 2007. Therefore the maximum storage time could be up to 290 days. Provide data supporting long term storage stability of DRSP in human plasma for at least 290 days. Alternatively, provide evidence that each sample from this study was analyzed for DRSP within 200 days of sample collection.
- The Sponsor replied on 2/3/2010 and stated that 62 samples from 4 different subjects (RNR 20, 28, 30, and 102) were out of the validated stability range of 200 days. Additionally, 8 samples from PK repeat reanalysis also exceeded the validated stability range. However, these repeat analysis results confirmed the original values, and therefore were not used for PK or BE analysis.
  - Subsequently, a memorandum from Division of Scientific Investigation (DSI) (DARRTS 3/2/2010) stated that there were 120 samples (instead of 70) that were stored for more than 200 days prior to analysis. The Sponsor clarified that the discrepancy was due to errors made by the bioanalysis contractor, (b)(4) (Submitted on 5/7/2010). A request was made and Sponsor provided a letter from (b)(4) stating that they had made errors and that the report on samples analyzed outside of proven stability by Sponsor is correct (Submitted on 5/27/2010).
  - The sponsor agreed to exclude samples analyzed outside of proven stability in the BE analysis.

### **Assay performance issues:**

#### EE and DRSP assays:

A DSI consult was sent (DARRTS, date 10/21/2009) for inspection of the clinical study site and bioanalysis sites for DRSP, EE, and MTHF for BE study 309664. DSI inspection report for the bioanalysis site for DRSP and EE was received on 3/2/2010 (Please see DSI memorandum in DARRTS, date 3/2/2010). The DSI inspection found several deficiencies and recommended the following actions:

1. The firm should establish new calibration curves for ethinylestradiol from 4 pg/mL to 1000 pg/mL and re-calculate all subject concentrations using the new calibration curves with 4 pg/mL as LLOQ (See 483 Item 1). The reviewer should evaluate re-calculated ethinylestradiol concentrations using the new calibration curves and exclude any concentration below 4 pg/mL for bioequivalence assessment.
2. The firm should establish new calibration curves for drospirenone from 0.5 ng/mL to 100 ng/mL and re-calculate all subject concentrations using the new calibration curve with 0.5 ng/mL as LLOQ. The reviewer should evaluate drospirenone concentrations using the new calibration

curves and exclude any concentration below 0.5 ng/mL for bioequivalence assessment. (See 483 Item 1).

3. The reviewer should exclude data for drospirenone from run AQ007-03 (Subjects 5 and 6 (Period 2 and 3)) (See 483 Item 3) and AQ06-008 (Subject 27 (Period 1 and 2) and Subject 31 (Period 1 and 3)) (See 483 Item 4) in bioequivalence evaluation.

4. The firm should evaluate the interference of blank reagent and matrix sample as well as the interference of the response of STD A and B prepared with the same matrix (10 5038) in all runs post September 24, 2007. If the observed interference was more than 20% of the response of STD A and B in any run, the firm should reject this run as in the firm's response (dated 2/16/2010) and provide reanalysis of the result in their amendment (See 483 Item 5) .

5. The reviewer should exclude data for drospirenone in the samples analyzed outside of 200-days long term frozen stability established in the validation experiment (See 483 Item 7).

This reviewer concurred with DSI's recommendations. The following recommendations were forwarded to the Sponsor on 3/31/2010.

Based on the findings of the FDA DSI inspection of [REDACTED] <sup>(b)(4)</sup>, we have the following recommendations regarding the bioanalysis of samples from Study 309664:

1. Establish new calibration curves for ethinyl estradiol (EE) from 4 pg/mL to 1,000 pg/mL and re-calculate all sample concentrations using the new calibration curves with 4 pg/mL as the lower limit of quantitation (LLOQ). Bioequivalence (BE) assessment should not include any EE concentration below 4 pg/mL.
2. Establish new calibration curves for drospirenone (DRSP) from 0.5 ng/mL to 100 ng/mL and re-calculate all sample concentrations using the new calibration curve with 0.5 ng/mL as the LLOQ. BE assessment should not include any DRSP concentration below 0.5 ng/mL.
3. For BE evaluation, exclude data for DRSP from runs AQ007-03 (calibration standard failed acceptance criteria), AQ06-008 (calibration standards processed [extracted] separately from the study samples and QCs), and AQ14-003 (chromatographic interference exceeding 20% of LLOQ).
4. For BE evaluation, exclude data for DRSP from samples analyzed outside of the 200 day long-term frozen stability established in the validation experiment. Clarify how many samples were analyzed outside of the validated stability of 200 days. The report should include a table of all samples and their associated duration of long term storage prior to bioanalysis.
5. Re-evaluate the interference of blank reagent and matrix sample as well as the interference of the response of STD A and B in all runs post September 24, 2007 (these were prepared using plasma ID 5038). If the observed interference was more than 20% of the response of STD A and B in any run, reject that run.

Upon completing the recommended corrective actions, the new data set should be assessed to determine if the data are adequate to permit calculation of bioequivalence (e.g., Are there samples missing that would prevent adequate calculation of individual pharmacokinetic parameters? Is there a sufficient number of subjects remaining?). If the data set is deemed acceptable for bioequivalence assessment, BE analysis should be performed. The results of the BE analysis, PK

profiles and calculated PK parameter values should be included as an amendment to Study Report A28575 and submitted to the NDA. The raw data set should also be submitted to the NDA in SAS Transport (.xpt) format. Submit the revised results and data files as soon as possible.

The Sponsor submitted a reply on 5/7/2010 and proposed that run AQ14-003 should be considered valid since it met all run acceptance criteria after LLOQ for DRSP was raised as per FDA's recommendation. This reviewer concurs. The Sponsor agreed to all other recommendations. A new study report for study 309664 incorporating these changes was submitted on 5/7/2010.

L-5-MTHF assay:

DSI recommended that L-5-MTHF data be accepted for review (DSI memorandum, DARRTS 5/24/2010).

Clinical site conduct for study 309664:

DSI noted that study record does not allow for verification of the treatment given in each period (DSI memorandum, DARRTS 5/24/2010). However, the treatment administered could be verified based on presence or absence of drug concentration. All subjects in treatment Beyaz or Yaz treatment periods had PK profile for EE and DRSP that is indicative of drug administration. Therefore, Metafolin mono tablet was not administered in these periods. To distinguish between Beyaz and Yaz treatment periods, assessment of L-5-MTHF concentration profile were conducted. For all subjects in period treated with Beyaz, their L-5-MTHF concentration-time profiles exhibited clear peak trough fluctuation in a pattern that is indicative of drug dosing. Furthermore, subjects in period treated with Yaz had low baseline-corrected L-5-MTHF concentrations and the concentration-time profiles were not consistent of exogenous drug administration. The above observations indicate that the subjects were administered the correct treatment in each period.

2.6.2 What bioanalytical methods were used to assess concentrations of RBC and plasma folate in pharmacodynamic studies 310662 (Yaz vs. Beyaz) and 309763 (Yasmin + folic acid vs. Yasmin + Metafolin)? Were these methods adequately validated?

Total folate in plasma and whole blood concentration from PD studies 309763 (Yasmin + Metafolin) and 310662 (Beyaz) were measured using a microbiological assay with *Lactobacillus casei*. The assay had an effective lower limit of quantitation of 75 ng/mL in whole blood and 3 ng/mL in plasma.

The assay was validated by (b) (4) (validation report A32903, (b) (4)). The assay had a calibration range of 25 – 500 ng/ml (56.64 – 1132.76 nmol/L) in blood and 1 – 20 ng/mL (2.27 – 45.31 nmol/L) in plasma. The validation report stated that the assay is not robust. Many plates were rejected due to calibration and/or quality control (QC) samples not meeting acceptance criteria. In some runs the calibration curve flattens at higher concentration and causing high bias for the High QC samples.

QC at LLOQ did not meet  $\pm 20\%$  for accuracy and precisions. The bias in accuracy at LLOQ was -21.8% in whole blood and -23.3% in plasma. Intra run imprecision at LLOQ was 19.4% in whole blood and 22.7% in plasma. Inter run imprecision at LLOQ was 27.6% in whole blood and 36.3% in plasma. Accuracy and precision criteria were met at other QC levels including QC Low (i.e., 75 ng/mL for whole blood and 3 ng/mL for plasma). The sponsor accepted the LLOQ with the expectation that study samples would not be below the QC Low level. Review of the datasets from studies 309763 and 310662 by statistics reviewer, Dr. Sonia Castillo, indicated that there were a limited number of samples that were below the effective LLOQ. These samples will be excluded from analysis.

Selectivity against DRSP and EE was evaluated. The assay was selective against DRSP and EE. Sensitivity for PGA (folic acid), 5-MTHF (racemic mixture), and effect of depletion of total folate by

folate binding protein (FBP) were also evaluated. The results indicated that the assay could accurately detect spiked PGA and 5-MTHF. Depletion by FBP was inconclusive but was suggestive that FBP could deplete folate.

Based on the validation results alone, absolute accuracy of observed in vivo study sample could not be assured since the QC low and QC medium were prepared in 5% bovine serum albumin (BSA) and not the true matrix (i.e., blood or plasma). QC high was based on spiked matrix and was acceptable. All QC samples used during bioanalysis of studies 309763 and 310662 were prepared in 5% BSA. At the request of DSI, the bioanalysis firm (b)(4) conducted additional accuracy test for spiked plasma and blood samples. DSI stated that (b)(4) has demonstrated sufficient accuracy for assays of folate in plasma and whole blood, except for folate determination of 3 ng/mL spiked into plasma." DSI recommended that plasma folate results below 3 ng/mL be omitted from analysis (DSI memorandum in DARRTS date 6/30/2010). Therefore, it could be concluded that there was sufficient accuracy at the "effective LLOQ" of 3 ng/mL in plasma and 75 ng/mL in blood.

Freeze/thaw stability in blood and plasma was established for 3 freeze/thaw cycles. Storage stability at <-70°C was acceptable for up to 3 years 2 months (based on additional data provided to DSI as documented in DSI memorandum on 5/24/2010). Blood samples could be diluted up to 5-fold and plasma samples could be diluted up to 100-fold.

#### **DSI inspection recommendations (DARRTS, 5/24/2010):**

The DSI inspected 2 clinical sites and the bioanalytical sites relating to PD studies 309763 and 310662. A team meeting was held on 6/28/2010 with representative from Clinical Pharmacology, DSI, Clinical, and Biostatistics discipline to discuss the issues raised by DSI. The following section lists the DSI recommendations that required a response and this reviewer's response, with concurrence from the review team.

#### Clinical Site Observations

A. Subjects identified using concomitant medication listed in the exclusion criteria should be excluded from analysis (Subjects #026 and 003) (See 483 Item 1 Coastal Carolina Research Center).

**Response:** Defer to Clinical reviewer.

B. Failure to protect samples from light exposure may have compromised folate stability. The identified samples should be excluded from further analysis. Note, only 60 of the 121 subjects at the investigated site were reviewed so the list of samples exceeding recommended light exposure may be incomplete (See 483 Item 1 Medical Center for Clinical Research).

**Response:** This observation was made at Medical Center For Clinical Research. The time from blood draw to RBC folate processing ranged up to 4 hours 3 minutes in the subjects investigated. In response to DSI's observation (attachment YEG2 in DSI's memorandum on 5/24/2010), Dr. William Koltun of Medical Center For Clinical Research agreed that they did not follow the instruction to place the blood samples for RBC analysis in the dark within 5 minutes after blood draws. A table provided by Dr. Koltun showed that about 64% of all samples at his site were processed after 5 minutes. This reviewer agrees that the identified samples should be excluded. The sponsor should identify and exclude all blood samples that were processed after 5 minutes. Alternatively, the sponsor can provide data to show that extended exposure to light do not alter the stability of folates in blood.

*Review note: The Clinical Team decided to exclude all data from this site due to other reasons. Therefore, this issue will not be pursued further. Please see Clinical review for details.*

#### Analytical Site Observations

A. Long-term frozen stability evaluations for whole blood are not adequate for the period of study sample storage. The High- and Medium-concentration QCs reported at 2 years, 11 months are outside the study sample acceptance criterion of  $\pm 15\%$  from nominal. Additionally, the potential changes in folate recovery and concentrations during the first freeze/thaw cycle have not been evaluated. The Review Division should request whole blood folate stability data for approximately 2 years, and during the first freeze/thaw cycle.

#### **Response:**

Long term stability: Table 11 shows the results of long-term stability data as provided by (b) (4) (i.e., the firm that conducted the bioanalysis of PD studies 310662 and 909763) to DSI (attachment SYK2 in DSI memorandum on 5/24/2010). The failed stability samples were at nominal concentrations of 200 and 400 ng/mL in phosphate buffered saline with bovine serum albumin (PBS-BSA). Both 400 ng/mL samples showed a recovery of 118%, slightly outside of the typical acceptance range of  $\pm 15\%$ . The 200 ng/mL samples showed a recovery of 129% and 113%, respectively. One was within the acceptable range while the second sample failed on the high side. Other tested stability samples in PBS-BSA, blood, and plasma were within the  $\pm 15\%$  criterion. Also, other stability data showed that total folate was stable for up to 10 months. A discussion with the Clinical team indicated that a precision of  $\pm 30\%$  would not be of a concern. Therefore, considering that the failed stability samples failed on the high side, other stability samples all passed, total folate samples were considered stable at up to 10 months, and the lack of need for a highly precise estimate of plasma and blood folate concentrations in the PD studies, this reviewer recommends that the samples be considered acceptable from a long term stability perspective. This recommendation was discussed at the review team meeting on 6/28/2010.

**Table 11: summary of long term stability data submitted by (b) (4) to DSI (from attachment SYK2 in DSI memorandum on 5/24/2010)**

QC	Matrix	result 1 ng/ml	result 2 ng/ml	avg	%sd	nominal ng/ml	recovery of nominal		Time interval
							recovery1	recovery2	
7002 QC endo	Blood	92.6	100.5	96.6	5.8%	endo	n.a.	n.a.	3 year 2 months
7002 QC high	Blood	316.0	317.0	316.5	0.2%	endo+ 200	110%	110%	3 year 2 months
7227 QC High	PBS-BSA	470.9	471.6	471.2	0.1%	400	118%	118%	2 year 11 months
7227 QC medium	PBS-BSA	257.9	226.7	242.3	9.1%	200	129%*	113%	2 year 11 months
7227 QC low	PBS-BSA	79.6	81.9	80.7	2.1%	75	106%	109%	2 year 11 months
7002 QC endo	Plasma	12.8	11.0	11.9	11%	endo	n.a.	n.a.	3 year 2 months
7002 QC high	Plasma	21.1	20.3	20.7	2.7%	endo+ 8	115%	105%	3 year 2 months
7002 QC endo	Plasma	11.9	11.5	11.7	2.4%	endo	n.a.	n.a.	3 year 6 months
7002 QC High	Plasma	20.7	20.2	20.4	1.7%	endo+ 8	112%	106%	3 year 5 months
7227 QC High	PBS-BSA	16.0	15.9	16.0	0.3%	16	100%	100%	2 year 11 months
7227 QC medium	PBS-BSA	8.1	7.8	7.9	3.2%	8	101%	97%	2 year 11 months
7227 QC low	PBS-BSA	3.1	3.0	3.0	2.5%	3	103%	99%	2 year 11 months

All QCs have been prepared twice, and each prepared sample was incubated in duplicate.

Result 1 = averaged result from a duplicate incubation in ng/ml. Result 2 = averaged result from a duplicate incubation in ng/ml.

Recovery = recovery of nominal value or nominal spike in %.

Recovery in Blood or Plasma is calculated as (measurement QC High - average measurements corresponding QC Endo)/nominal spike in %.

Recovery in PBS-BSA is calculated as measurement/nominal value in %. n.a.: not available, since QC Endo reflects the endogenous (unknown) concentration.

\*Slightly above the acceptance criterion (recovery between 80 and 120%).

Stability following the first freeze/thaw cycle: Assay validation was conducted such that all QC samples were prepared and stored at -70 °C prior to use. For freeze/thaw stability testing the sponsor compared the expected value based on mean of QC endo and QC high in the same assay run. Since the QC endo and QC high have been frozen before, the question remains whether there is an effect of the first freeze/thaw cycle (the effect of second and third freeze thaw cycles can be delineated from available data and do not show a difference between the first, second, and third cycles).

(b) (4) has provided additional data to DSI and DSI has concluded that there are sufficient stability of folate during the first freeze/thaw cycle (see DSI memorandum in DARRTS date 6/30/2010).

B. (b) (4) should evaluate the recovery of folate in plasma and whole blood at three concentrations to ensure the accuracy of study sample determinations. As mentioned, (b) (4) expected to provide these data by 5/15/10 but, due to technical issues, will provide the data by 5/31/10. In the absence of these data, the majority of the reported concentrations in the audited studies cannot be assured.

**Response:** (b) (4) has provided additional data to DSI. (b) (4) evaluated folate concentrations in whole blood samples spiked at 100, 200, and 300 ng/mL more than endogenous, and in plasma samples spiked at 3, 8, and 12 ng/mL more than endogenous. DSI stated that (b) (4) has demonstrated sufficient accuracy for assays of folate in plasma and whole blood, except for folate determinations of 3 ng/mL spiked into plasma. If the 115% acceptance criterion is used, DSI recommends that plasma folate results below 3 ng/mL should be omitted from analysis” (see DSI memorandum, date 6/30/2010). Since we have already used an “effective LLOQ” threshold of 3 ng/mL in plasma, no further action is needed.

C. In the absence of demonstration of dilution linearity for 8-fold diluted samples, the six diluted samples should be omitted from analysis.

**Response:** DSI memorandum on 6/30/2010 stated that additional data at 8-fold diluted did not meet acceptance criteria and the prior recommendation stands. This reviewer agrees that the six 8-fold diluted samples should be omitted from analysis.

D. The subject samples accepted with only 4 QCs (two concentrations each in duplicate) should be omitted from analysis. Samples identified by firm as approved outside the study plan should also be excluded.

**Response:**

Samples from runs with only 2 QC: the Sponsor accepted 2 series (Blood series 22, 3 out of 18 plates and Plasma series 8, 24 out of 24 plates) where the QC high was accidentally not pipetted into the plates and only the low and medium QCs were available for applying the acceptance criteria. The sponsor provided the following justifications:

- All the measured concentrations were within the calibrated range.
- For both series (series 22 for whole blood, series 8 for plasma, which represent all data mentioned in the observation), all 4 out of 4 remaining QCs were within specification (< 15% deviation).
- In addition, in each series of the study for whole blood and for plasma, the WHO International Standard Folate, (NIBSC code: 95/528) was included on each plate. The folate concentration in the reconstituted material is 13 ng/ml (if reconstituted to represent plasma) or 130 ng/ml (if reconstituted to represent whole blood). For all individual plasma plates, the accuracy for both duplicates of this material was within 85-115% (actual values 97-107%). For all individual whole blood plates, the accuracy for both duplicates of this material was within 85-115% (actual values 96-101%).
- For QC High in this study ( (b) (4) ), zero out of all 206 plasma plates, and only one out of a total of 270 whole blood plates failed the acceptance criteria.

The standard acceptance criteria are 1) at least 4 of 6 QC samples should be between  $\pm 15\%$  and 2) at least one of 2 QC should be valid at each level. Due to the absence of the high QC, the second acceptance criteria could not be met. However, based on the above observations that all remaining QC samples were within the  $\pm 15\%$  criteria and further supported by the WHO International Standard Folate duplicate samples being within  $\pm 15\%$  of their expected values, these runs could be considered acceptable. This recommendation was discussed at the review team meeting on 6/28/2010.

Samples approved outside the study plan: These samples should be excluded unless the sponsor can provide adequate justification.

2.6.3 What bioanalytical methods were used to assess concentration of folate metabolites?

Concentrations of PGA (folic acid), THF (tetrahydrofolate), 5-MTHF (5-methyl-THF), 5-FTHF (5-formyl-THF), and 5,10-MTHF (5,10-methyl-THF) from study 309763 were measured using a semi-quantitative LC/MS assay. The assay was based on acceptance criteria of 50% for accuracy and precision at LLOQ (40% at other QC levels). The calibration range was 0.5 – 150 ng/mL. The QC levels (LLOQ, QC Low, QC Medium, and QC High) were at 0.5, 1.5, 20, and 100 ng/mL for 5-MTHF, and 0.5, 1.5, 5, and 10 ng/mL for the remaining analytes.

The accuracy for THF failed to meet the acceptance criteria at initial validation runs. The sponsor noted that runs 2 and 3 conducted after a freeze/thaw cycle had lowed calculated concentration suggesting that THF was not stable. Subsequently, runs 2 and 3 were repeated (noted as runs 1a and 1b) with freshly prepared stocks and did meet the acceptance criteria for accuracy, except for the LLOQ sample at 0.5 ng/mL.

No data on storage stability was provided in the validation report. There were evidence that a freeze/thaw cycle may cause degradation of THF as observed concentrations in validation runs 2 and 3 (previously frozen samples) were lower as compared to those from run 1 (fresh samples).

Based on the original validation report the following conclusion was drawn: the data generated from this assay is not considered quantitative due to the wide acceptance criteria and the lack of storage and freeze/thaw stability data. The Clinical review team indicated that for their purpose, they would accept qualitative data on relative metabolite profiles. Therefore, it was decided to request from the sponsor information on storage and freeze/thaw stability to ensure that the samples were stable prior to analysis. The following request was sent to sponsor on 4/15/2010.

#### **Information request for sponsor:**

Clinical Study Report A34010 included a validation report (b) (4) and a bioanalysis report (b) (4) for determination of folate metabolites in human plasma from clinical study 309763. The validation report did not include the following stability assessments: freeze/thaw, short-term storage (e.g., at room temperature) and long-term storage (e.g., at minus 20°C or minus 70°C). In addition, it appears that at least 1 or 2 of the tested moieties (i.e., tetrahydrofolate [THF] and 5,10-methyl-THF [5,10-MTHF]) were not stable following a freeze/thaw cycle, as indicated by the results from validation Runs 2 and 3 compared to Run 1. Provide the following data as soon as possible:

1. Evidence of long-term storage stability for each tested moiety. In addition, provide a table showing the storage time (i.e., time from sample collection to time of analysis) for each study sample and whether or not it was analyzed within the demonstrated storage stability period. Samples analyzed outside of the demonstrated stability period should not be used.
2. Evidence of stability upon 3 freeze and thaw cycles for each tested moiety. In addition, provide a table showing how many freeze and thaw cycles each sample incurred prior to analysis. Given that all samples were stored at below minus 70°C upon receipt by (b) (4), all would have incurred at least 1 freeze and thaw cycle.
3. Evidence of short-term storage stability for each tested moiety. The duration of demonstrated short-term storage stability should be sufficient to cover the time allotted to process the study samples.

#### **Sponsor's response:**

The Sponsor provided a response to the above request on 7/16/2010. A summary of the response is outlined below for each request.

1. The sponsor stated that long term storage stability at -70 °C was demonstrated for folic acid for 38 months in whole blood and 41 months in plasma ( (b) (4) Long term stability at -80

°C for L-5-MTHF in serum was demonstrated for 552 days based on validation study performed in support of the BE study (b) (4). However, no stability data is available for the other metabolites (5,10-MTHF, 5-FTHF/10-FTHF, THF).

The sponsor stated that all bioanalyses for folic acid and L-5-MTHF were completed within the proven long-term storage stability range (as stated above). However, a similar conclusion can't be drawn for the other metabolites. Therefore, the results for metabolites 5,10-MTHF, 5-FTHF/10-FTHF, THF are not considered reliable.

2. The sponsor stated that freeze thaw stability over 3 freeze/thaw cycles for total folate in plasma and whole blood was demonstrated in (b) (4) (spiked folic acid samples analyzed with microbiological assay with *Lactobacillus casei* that is sensitive to total folate). Freeze thaw stability of L-5-MTHF over 3 freeze/thaw cycles was demonstrated in (b) (4).

The Sponsor also provided an analysis showing results of effect of 1 freeze thaw cycle showing that in addition to folic acid and L-5-MTHF, 5-FTHF was stable after 1 freeze thaw cycle (The majority of samples [481 out of 501] were thawed only once). It should be noted that this analysis by sponsor was based on 3 different sets of stability samples. All 3 sets were analyzed fresh but only one set were analyzed following a freeze thaw cycle. Additionally, this data was based on a semi-quantitative assay and do not provide clear evidence of freeze thaw stability. However, assuming the samples were prepared correctly, the data may be considered reliable for use in conjunction with the current semi-quantitative assay. The results of this analysis also suggested that 5,10-MTHF and THF were not stable after the freeze thaw cycle with mean recovery of 61% and 21%, respectively.

Overall, for purpose of use as it relate the current semi-quantitative assay for folate metabolites, the issue with freeze thaw stability for folic acid, L-5-MTHF, and 5-FTHF is considered resolved. The data suggests that 5,10-MTHF and THF may be unstable following a freeze thaw cycle and therefore the observed values may lower than the actual values.

3. Short term storage stability at room temperature up to 24 hours is available for total folate in whole blood and plasma and L-5-MTHF in serum. Short term storage stability for other metabolites was not established.

#### Overall assessment of the metabolite LC/MS assay:

This assay is a semi-quantitative assay. Available data supports the stability of folic acid and L-5-MTHF and bioanalytical results for these 2 moieties could be reviewed (taking into account the semi-quantitative nature of the assay). Stability of 5-FTHF, 5,10-MTHF, and THF were not established. Available data suggests that 5,10-MTHF and THF are not stable following a freeze thaw cycle. Therefore, bioanalytical results for 5-FTHF, 5,10-MTHF, and THF are not considered reliable.

#### **DSI inspection recommendations (DARRTS, 5/24/2010):**

The DSI inspected the bioanalytical sites relating to the folate metabolite assay for clinical study 309763. The following section lists the DSI recommendations and this reviewer's response.

A. The lack of audit trail during manual chromatogram reintegrations prevents reconstruction of the events, and therefore prevents assurance of the data. However, for semi-quantitative purposes of this assay (risk of neural tube defects; pharmacogenomics), these data may be usable.

**Response:** No comment.

B. The procedure for Sponsor-requested re-assays was not defined prior to study initiation. The original values should be used for pharmacokinetic assessments.

**Response:** Twenty-one (21) out of 485 samples were reanalyzed for “implausibility” at the request of sponsor. The median of the duplicate reassays and the original value was reported in (b) (4). Even though (b) (4) did not pre-specify the calculation method, the method used was a standard practice that is used at (b) (4). A review of the data indicated that the median values were generally consistent with the original values. The use of one set of values versus the other would unlikely to alter the conclusion from this study.

C. Documentation (especially dates of events) was incomplete for the preparation of calibrator and QC samples. However, the available records and data are consistent with preparation according to the documented procedure.

**Response:** No comment.

### 3 Detailed Labeling Recommendations

The labeling recommendations are outlined below. Specific labeling language will be communicated directly with the review team.

- The label should state that the effect of food on bioavailability of metformin was not evaluated.
- [REDACTED] (b) (4)
- The statement [REDACTED] (b) (4) in section 7.3 should be deleted.
- Literature information for folate in section 12.3 that are not well supported or not necessary for safe and effective use of Beyaz should be deleted.

## 4 Appendices

### 4.1 Individual Study Reviews

#### 4.1.1 Review of bioequivalence study report A28575 (Protocol 309664, Yaz) as amended on 5/5/2010

**Title:** Open-label, randomized, three-fold crossover study to investigate the bioequivalence of two different tablet formulations containing 0.02 mg EE and 3 mg DRSP without [SH T00186D, Yaz] and with [SH T04532B, Beyaz] 0.451 mg Metafolin, and to investigate the bioequivalence of two different tablet formulations containing 0.451 mg Metafolin without [SH T04532C, Metafolin only] and with 0.02 mg EE / 3 mg DRSP [SH T04532B] in 42 healthy young women.

**Objective:** To investigate 1) the BE of EE and DRSP in Yaz and Beyaz tablets, and 2) the BE of L-5-MTHF in Beyaz and Metafolin only tablets.

**Study design:** Single-center, open-label, randomized, cross-over study with three treatments (one dose each), three study periods separated by at least one menstrual cycle, and six different treatment sequences. Forty four healthy women aged 18 – 38 years of age (mean 26.3 years) with body mass index (BMI) of 19 – 28 kg/m<sup>2</sup> (mean 22.5 kg/m<sup>2</sup>) were enrolled (Full analysis set or FAS). Forty subjects completed the study (Per protocol set or PPS).

The study comprised six periods: Screening (visits 1 and 2), pre-dose, treatment period 1, treatment period 2, treatment period 3 and follow-up. A washout of at least one menstrual cycle occurred between treatment periods.

Each treatment was administered between the third and sixth menstrual cycle day. The study drug was administered after an over night fast (at least 10 hours) in the morning between 7:00 am and 10:00 am. The study drug was taken with 240 ml of water. Fasting is continued for 4 hours post drug administration.

A folate-free (<5 µg folate/100 g powder) shake (85 g of diet powder containing 1831 kJ, 4 g protein, 58 g carbohydrate, 21 g fat mixed with 240 ml water) was ingested between 3 to 2 hours before drug administration. To minimize folate intake, the same shake was also administered at 4, 7, and 10 hours post drug administration in lieu of regular food intake (i.e., lunch). The shake administration at 3 to 2 hours predose should not interfere with the interpretation of the BE results. Since the shake is administered as a liquid suspension, after 2 hours it should be emptied from the stomach. Additionally, this condition is applied to all treatment period.

**Study period:**

Date of first volunteer, first visit: 10/20/2006

Date of last volunteer, last visit: 9/13/2007

**Test products:**

Treatment A: Single oral administration of 1 coated tablet SH T00186D (Yaz) containing 0.020 mg EE and 3 mg DRSP

Treatment B: Single oral administration of 1 coated tablet SH T04532B (Beyaz) containing 0.020 mg EE, 3 mg DRSP, and 0.451 mg Metafolin

Treatment C: Single oral administration of 1 coated tablet SH T04532C (Metafolin) containing 0.451 mg Metafolin

**PK samplings:**

Samples for assessment of serum L-5-MTHF, and plasma EE and DRSP were obtained at the time points indicated in the table below. EE and DRSP were not measured in Treatment C.

**Table 12: PK sampling times**

Time (h)	- .5	.5	1	1.5	2	3	4	6	8	10	12	16	24	34	48	72	96	12	144	168
L-5-MTHF	x	x	x	x	x	x	x	x	x	x	x									
EE	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
DRSP	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			

**Bioanalytical analysis:**

The concentration of EE and DRSP in plasma samples and L-5-MTHF in serum samples was determined by a validated liquid chromatography / tandem mass spectrometry (LC/MS/MS) with a lower limit of quantification (LLOQ) of 4 pg/ml (EE), 500 pg/ml (DRSP) and 1.09 nmol/L (L-5-methyl-THF). Results were reported in Bioanalytical Report number A34097.

The Division of Scientific Investigation has made several recommendations, including raising the LLOQ for EE and DRSP assays, exclusion of samples analyzed outside of the validated stability for DRSP, and exclusion of select runs (see discussion within the Question Based Review for further details). The Sponsor has performed the recommended corrective actions and the study report for study 309664 was amended.

**Statistical analysis:**

The primary BE variables are C<sub>max</sub> and AUC. In cases where AUC could not be reliably calculated, the AUC<sub>0-tlast</sub> would be considered the primary variable instead of AUC. This was the case for EE and L-5-MTHF.

90% confidence intervals were calculated for Treatment A vs. Treatment B (bioequivalence of EE and DRSP) and Treatment B vs. Treatment C (bioequivalence of L-5-MTHF).

**Protocol Deviations:**

There were 4 major protocol deviations. These were not considered to have an adverse effect on the study outcome.

For subject number 16, the study medication for period 3 was erroneously administered in period 2. She was allowed to continue in the study with administration of the medication for period 2 in period 3. This volunteer was moved from sequence group 6 to sequence group 5. This should not adversely affect the BE results and is acceptable.

**PK results:**

EE:

For Beyaz hormone tablets, the mean (±SD) EE C<sub>max</sub> was 44.1 ± 14.1 pg/mL and was reached 1.52 hours (range: 1 to 4.2 hr) after administration. Mean values for AUC(0-tlast) was 390 ± 129 pg\*h/mL. The AUC and t<sub>1/2</sub> were 451 ± 167 pg\*h/mL and 10.4 ± 3.70 hr respectively (table 13). The AUC and t<sub>1/2</sub> could be determined only for 8 volunteers, due to insufficient time range for half-life calculation of less than two half-lives and/or an extrapolated part of the AUC greater than 20% or due to the exclusion of subjects.

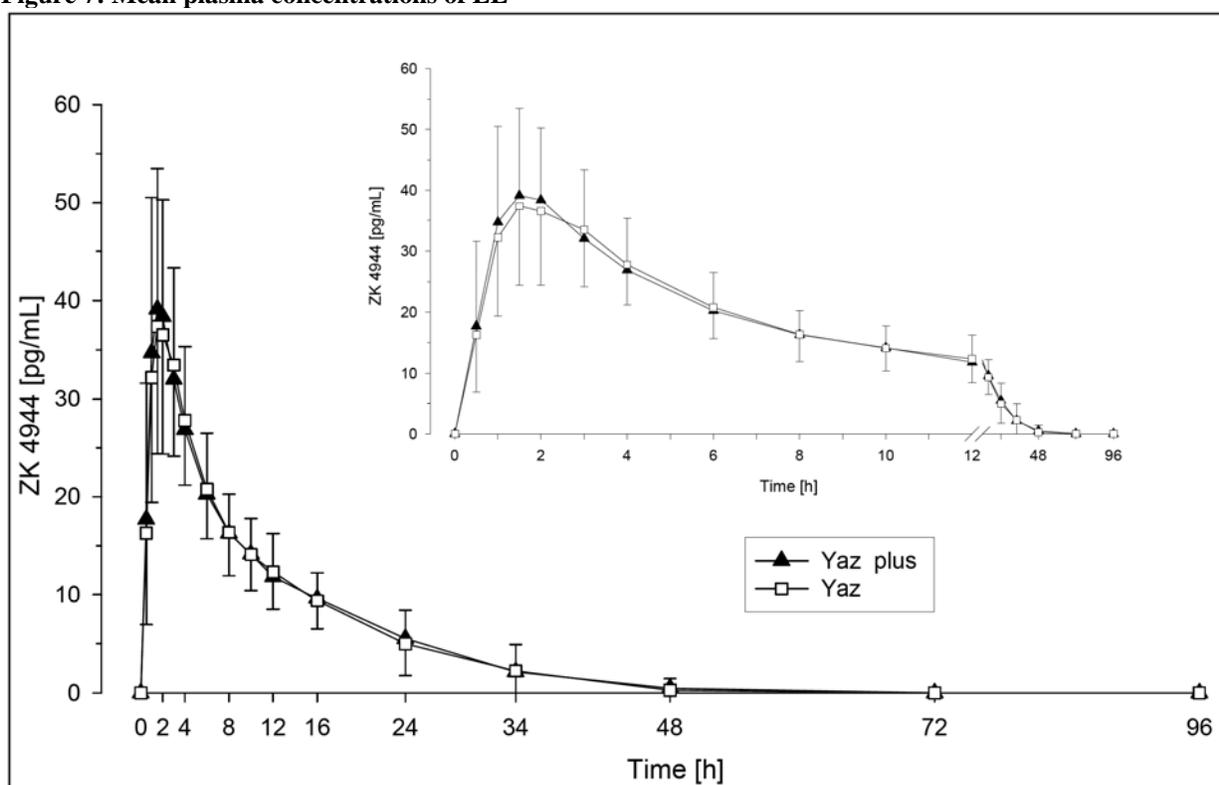
The mean PK profiles for Beyaz hormone and Yaz hormone tablets were similar (figure 7). The results of BE analysis showed that the 90% CI of Beyaz/Yaz ratio for EE  $C_{max}$  and AUC were within the 80 – 125% limits, indicating that Beyaz and Yaz were BE with respect to EE.

**Table 13: Arithmetic mean ( $\pm$  SD) pharmacokinetic parameters of EE**

Treatment	Parameter				
	$C_{max}$ (pg/mL)	$T_{max}^*$ (h)	$T_{1/2}$ (h)	AUC (pg*h/ml)	AUC(0-tlast) (pg*h/ml)
Beyaz (N=39)	44.1 $\pm$ 14.1	1.52 (1 - 4.18)	10.4 $\pm$ 3.70 (N=8)	451 $\pm$ 167 (N=8)	390 $\pm$ 129
Yaz (N=39)	41.6 $\pm$ 13.1	1.5 (0.5 - 4)	9.71 $\pm$ 3.76 (N=14)	452 $\pm$ 155 (N=14)	379 $\pm$ 136

\* = Median (range)

**Figure 7: Mean plasma concentrations of EE**



Yaz plus = Beyaz

**Table 14: Bioequivalence assessment for EE**

EE	N	Yaz (reference)	Beyaz (test)	Test/reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [pg*h/ml]	38	379.0	388.7	103.5	(99.2, 108.0)
$C_{max}$ [pg/mL]	38	41.7	44.1	106.0	(98.9, 113.7)

Note that for the geometric means and bioequivalence assessment of AUC(0-tlast), only data from 38 subjects was used (data for both reference and test available). RNR 39 and RNR 40 had missing values.

DRSP:

For Beyaz hormone tablets, the mean ( $\pm$ SD) DRSP  $C_{max}$  in Beyaz was  $27.6 \pm 6.97$  ng/mL and was reached 2 hours (range: 0.5 to 4.1 hr) after administration. Mean values for AUC(0–tlast) was  $400 \pm 125$  ng\*h/mL. The AUC and  $t_{1/2}$  were  $437 \pm 137$  ng\*h/mL and  $31 \pm 9.14$  hr, respectively (table 15).

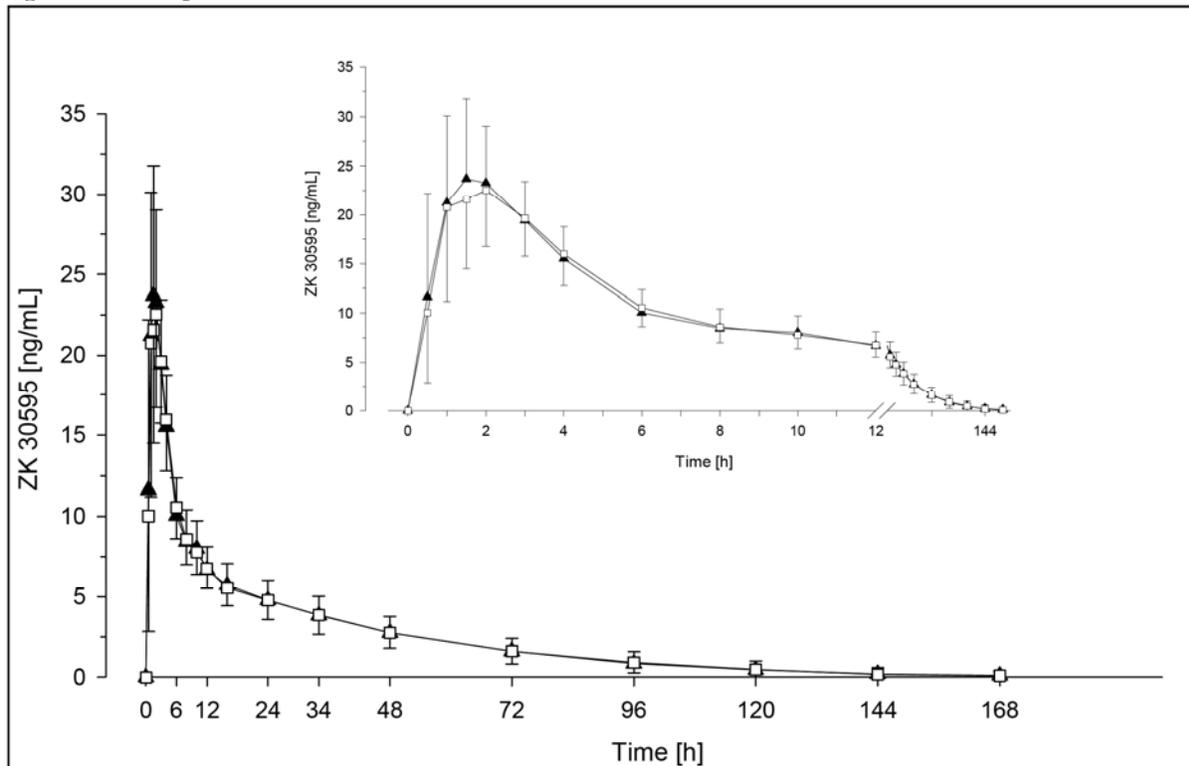
The mean DRSP PK profiles for Beyaz hormone and Yaz hormone tablets were similar (figure 8). The results of BE analysis showed that the 90% CI of Beyaz/Yaz ratio for DRSP  $C_{max}$  and AUC were within the 80 – 125% limits, indicating that Beyaz and Yaz were BE with respect to DRSP.

**Table 15: Arithmetic mean ( $\pm$  SD) pharmacokinetic parameters of DRSP**

Treatment	Parameter				
	$C_{max}$ (pg/mL)	$T_{max}^*$ (h)	$T_{1/2}$ (h)	AUC (pg*h/ml)	AUC(0-tlast) (pg*h/ml)
Beyaz (N=36)	$27.6 \pm 6.97$	2 (0.5 – 4.1)	$31.0 \pm 9.14$ (N=33)	$437 \pm 137$ (N=33)	$400 \pm 125$
Yaz (N=35)	$26.2 \pm 6.44$	2 (0.5 – 4)	$31.3 \pm 8.27$ (N=34)	$437 \pm 115$ (N=34)	$400 \pm 110$

\* = Median (range)

**Figure 8: Mean plasma concentrations of DRSP**



Open squares: Yaz, closed triangles: Beyaz

Note that for bioequivalence assessment, only data from 33 subjects was used (data for both reference and test available).

**Table 16: Bioequivalence assessment for DRSP**

DRSP	N	Yaz (reference)	Beyaz (test)	Test/reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [pg*h/ml]	34/35	396.8	397.9	100.5	(97.3, 103.8)
Cmax [pg/mL]	34/35	26.3	27.7	105.2	(97.3, 113.8)

Note that for bioequivalence assessment, only data from 33 subjects was used (data for both reference and test available).

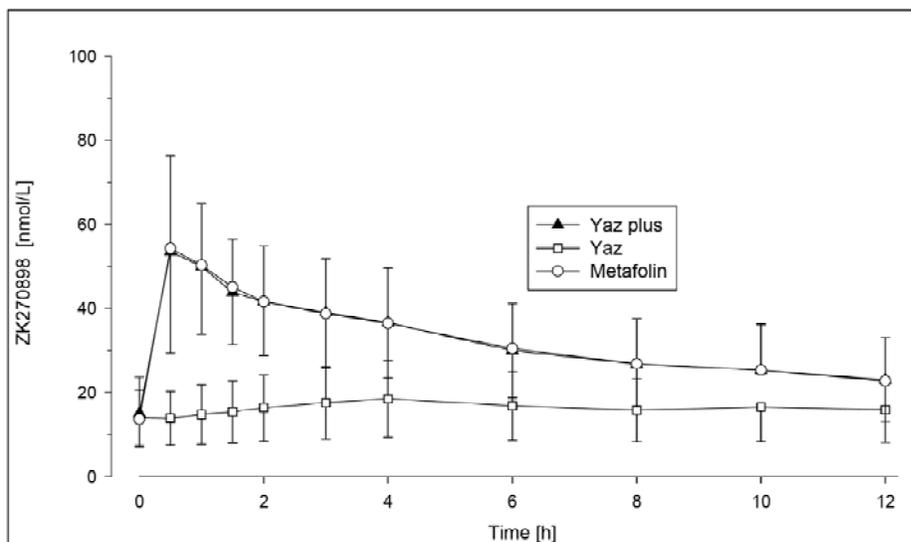
**L-5-MTHF:**

Table 17 shows the PK parameters (both baseline-corrected and baseline-uncorrected) following single dose administration of Beyaz and Metafolin mono tablets. Bioavailability was similar between the 2 formulations.

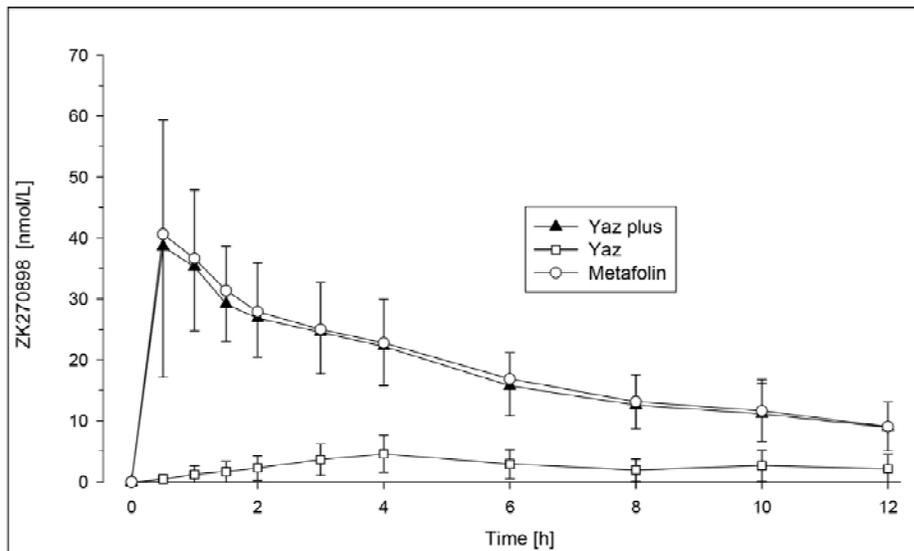
**Table 17: Arithmetic mean ( $\pm$ SD) L-5-MTHF PK parameters (Study 309664)**

Baseline method	Treatment	C <sub>max</sub> (nmol/L)	AUC <sub>0-tlast</sub> (nmol/L*h)	T <sub>max</sub> * (h)	T <sub>1/2</sub> (h)
Baseline - uncorrected	Beyaz (n=40)	60.4 $\pm$ 18.0	391 $\pm$ 137	0.5 (0** - 4)	NC
	Metafolin mono (n=40)	61.2 $\pm$ 21.8	391 $\pm$ 137	0.5 (0.5 - 2)	NC
Baseline - corrected	Beyaz (n=39)	46.5 $\pm$ 14.3	222 $\pm$ 63	0.5 (0.5 - 4)	4.68 $\pm$ 0.474
	Metafolin mono (n=40)	47.5 $\pm$ 18.9	226 $\pm$ 68	0.5 (0.5 - 2)	4.54 $\pm$ 0.504

\* T<sub>max</sub> is shown as median and range.  
 \*\* One subject (RNR 004) had T<sub>max</sub> at predose time point  
 NC Not calculated

**Figure 9: Mean serum concentration ( $\pm$ SD) of baseline-uncorrected L-5-MTHF**

**Figure 10: Mean serum concentration ( $\pm$ SD) of baseline-corrected L-5-MTHF**



**Table 18: Bioequivalence assessment for L-5-MTHF (baseline-corrected, PPS)**

L-5-methyl-THF	N	Metafolin (reference)	Beyaz (test)	Relative bioavailability (test / reference) in %	Two-sided 90% confidence interval in %
AUC <sub>(0-tlast)</sub> [nmol*h/l]	40 / 39	225.76	222.80	98.16	( 93.95 , 102.57 )
C <sub>max</sub> [nmol/l]	40 / 39	47.46	46.50	99.88	( 91.24 , 109.34 )

Note: ANOVA was based on 39 complete data sets

**Table 19: Bioequivalence assessment for L-5-MTHF (baseline-uncorrected, PPS)**

L-5-methyl-THF	N	Metafolin (reference)	Beyaz (test)	Relative bioavailability (test / reference) in %	Two-sided 90% confidence interval in %
AUC <sub>(0-tlast)</sub> [nmol*h/l]	40	390.99	391.07	99.88	( 95.38 , 104.58 )
C <sub>max</sub> [nmol/l]	40	61.19	60.40	100.28	( 93.15, 107.95 )

**Conclusions:**

BE for DRSP and EE were demonstrated between Beyaz and Yaz. BE for L-5-MTHF was demonstrated between Beyaz tablet (SH T04532B) and the Metafolin only tablet (SH T04532C).

## 4.2 Pharmacogenomics Review

Note: The Pharmacogenomics review refers to Beyaz as Yaz plus or Yaz +.

**CLINICAL PHARMACOLOGY GENOMICS GROUP REVIEW**

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<b>NDA/BLA Number</b>	NDA 022532
<b>Submission Type; Code</b>	NME
<b>Applicant Name</b>	Bayer HealthCare Pharmaceuticals Inc.
<b>Submission Date</b>	Aug 21 2009
<b>Brand Name</b>	YAZ Plus
<b>Generic Name</b>	drospirenone, ethinyl estradiol, and levomefolate calcium
<b>Proposed Indication</b>	Oral contraceptive for birth control for the: <ul style="list-style-type: none"> <li>• prevention of pregnancy;</li> <li>• treatment of symptoms of premenstrual dysphoric disorder (PMDD);</li> <li>• treatment of moderate acne for women of at least 14 years old;</li> <li>• improvement in folate status</li> </ul>
<b>Genomics Reviewer</b>	Li Zhang, PhD
<b>Team Leader</b>	Issam Zineh, PharmD, MPH

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## 1. BACKGROUND

YAZ Plus is submitted as drospirenone (DRSP) 3mg/ethinyl estradiol (EE) 0.02mg/levomefolate calcium 0.451mg oral tablets for the additional indication of improvement in folate status in women who elect to use an oral contraceptive (OC). YAZ is a combination oral contraceptive containing 3mg of DRSP and 0.02mg of EE stabilized by beta-cyclodextrin(betadex) as a clathrate. YAZ has been approved for three indications: 1) prevention of pregnancy in women who elect to use an oral contraceptive (NDA21676); 2) treatment of symptoms of premenstrual dysphoric disorder (PMDD) (NDA 21873); 3) treatment of moderate acne vulgaris in women at least 14 years of age, who have no known contraindications to oral contraceptive therapy and have achieved menarche. YAZ should be used for the treatment of acne only if the patient desires an oral contraceptive for birth control (NDA22045). YAZ Plus includes the additional component of Metafolin (levomefolate calcium) to YAZ and is intended to be a folate-fortified OC regimen.

## 2. NDA CONTENT RELATED TO GENOMICS

Multicenter, randomized, double-blind, active-controlled, parallel-group study was submitted to investigate plasma folate, red blood cell folate and homocysteine levels during a 24-week oral administration of an OC containing metafolin (Yaz+) compared to OC alone (Yaz). Efficacy evaluations include RBC and plasma folate levels at Week 24 (Cycle 6), the mean of the neural tube defect (NTD) risk reduction, the mean changes from baseline in RBC folate, plasma folate, and plasma homocysteine. Genetic 5,10-methylenetetrahydrofolate reductase (MTHFR) 677 C>T descriptive statistics were provided but no genetic data were provided in the submission.

## 3. KEY QUESTIONS AND SUMMARY OF GENOMICS FINDINGS

### 3.1. Are MTHFR variants associated with differential effects of Yaz+ on folate biomarkers?

Sponsor conducted the study in 4 phases: Screening (Visit 1 and Visit 2), Baseline (Visit 3), and Blinded Treatment Phase (Visits 4, 5, 6, 7, 8, and 9) for 24 weeks, and Follow-up/End of Study Phase (Visit 10, Weeks 26 through 28). Plasma MTHFR polymorphisms and 3 other exploratory gene polymorphisms were tested (Table 1).

Table 1:

DNA polymorphisms:							
Gene	Position mRNA	Reference sequence (wild type) <sup>a</sup>	Variant (mutation) <sup>a</sup>	Position Protein	Wt	Mutation	dbSNP
MTHFR	677	C	T	222	A	V	rs1801133
MTHFR	1415	A	C	429	E	A	rs1801131
SLC19A1	80	A	G	27	H	R	rs1051266
MTHFD1	1958	G	A	652	R	Q	rs2236225
DHFR	IVS1+59	ACCTGGGCG GGACGCGCCA					

The MTHFR 677 C>T is the most established polymorphism related to folate metabolism. The 677 C>T polymorphism in the MTHFR gene has been reported to interact with folate status in determining elevated total plasma levels of homocysteine. Overall, the CC genotype (wild

type) and heterozygote (CT) genotype were detected in a comparable proportion of subjects. The occurrence of the CT genotype was the same for the YAZ Plus (46.4%) and YAZ treated (46.0%) subjects. The CC genotype occurred more often for YAZ (50.8%) compared with YAZ Plus (41.8%) subjects. Whereas the TT genotype occurred in 23 (11.7%) of the YAZ Plus subjects, only 2 subjects (3.2%) presented that genotype in the YAZ group. The frequency of these genotypes for the MTHFR 677 C>T polymorphism and the other polymorphisms included for exploratory reasons is presented in Table 2. Fisher-Exact tests show C/T frequencies in both YAZ Plus and YAZ were under Hardy-Weinberg equilibrium (HWE). The distribution of genotype frequencies in the study population was consistent with what has been reported in the literature.

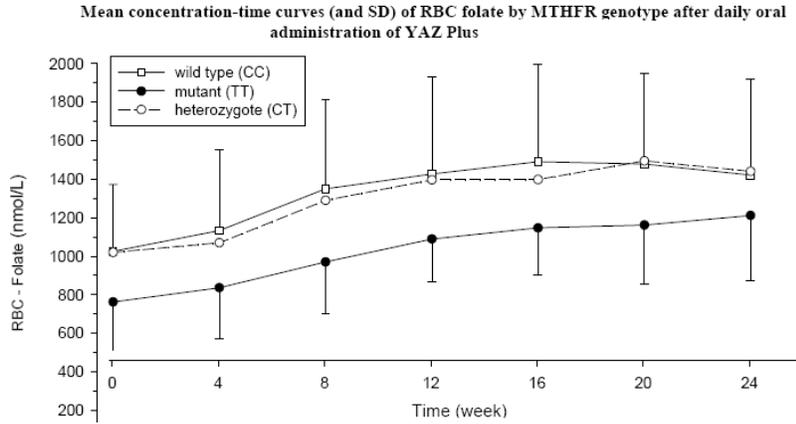
Table 2:

Frequency of genetic polymorphisms by treatment group (Per Protocol Set)				
Polymorphism	Genotype	YAZ Plus (N = 196) n (%)	YAZ (N = 63) n (%)	Overall (N = 259) n (%)
MTHFR 677 C>T	wild type (CC)	82 (41.8)	32 (50.8)	114 (44.0)
	heterozygote (CT)	91 (46.4)	29 (46.0)	120 (46.3)
	Mutant (TT)	23 (11.7)	2 (3.2)	25 (9.7)
MTHFR 1415 A>C	wild type (AA)	113 (57.7)	30 (47.6)	143 (55.2)
	heterozygote (AC)	73 (37.2)	28 (44.4)	101 (39.0)
	Mutant (CC)	10 (5.1)	5 (7.9)	15 (5.8)
MTHFD1 1958 G>A	Missing	0 (0.0)	1 (1.6)	1 (0.4)
	wild type (GG)	85 (43.4)	23 (36.5)	108 (41.7)
	heterozygote (GA)	70 (35.7)	26 (41.3)	96 (37.1)
	Mutant (AA)	41 (20.9)	13 (20.6)	54 (20.8)
SLC19A1 80 A>G	wild type (AA)	53 (27.0)	15 (23.8)	68 (26.3)
	heterozygote (AG)	80 (40.8)	33 (52.4)	113 (43.6)
	Mutant (GG)	63 (32.1)	15 (23.8)	78 (30.1)
DHFR IVS1+59	wild type (ref/ref)	30 (15.3)	14 (22.2)	44 (17.0)
	heterozygote (ref/ins)	106 (54.1)	30 (47.6)	136 (52.5)
	Mutant (ins/ins)	60 (30.6)	19 (30.2)	79 (30.5)

### MTHFR 677 C>T:

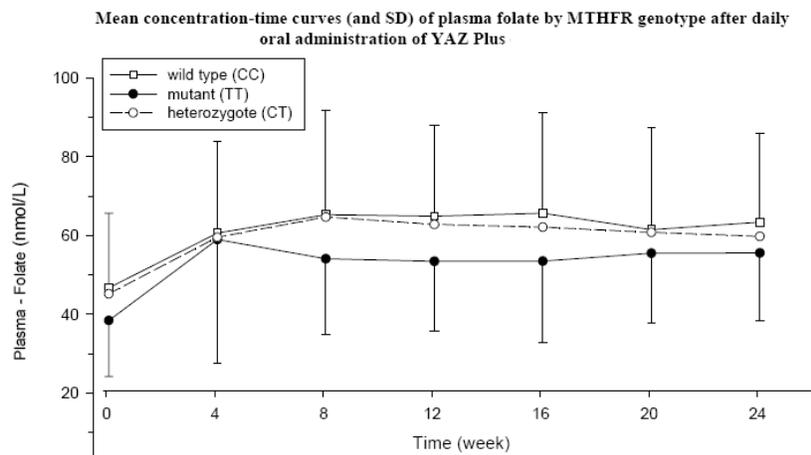
Baseline mean ( $\pm$  standard deviation) RBC folate levels were comparable for the wild type (CC) and heterozygote (CT) genotypes in both the YAZ Plus and YAZ treatment groups. Mean RBC folate levels were lowest for the mutant (TT) genotype group ( $763 \pm 260$  nmol/L) for the YAZ Plus treatment group (Figure 1). For YAZ Plus, mean RBC folate levels of all genotype groups increased steadily through the first 16 weeks after treatment initiation and then reached a mean steady state concentration for the remainder of the study (Figure 1). In the YAZ group, only minor changes of the mean RBC folate levels  $\pm$  SD were observed for the CC and the CT genotype group between baseline and the treatment phase. In the YAZ Plus group at Week 24, the mean change from baseline was comparable between all 3 genotype groups. For the TT genotype, a mean change from baseline of  $444 \pm 150$  nmol/L was observed for Week 24 compared with  $438 \pm 416$  and  $396 \pm 306$  nmol/L for the CT and CC genotypes, respectively.

Figure 1:



Baseline mean ( $\pm$  SD) plasma folate levels at were comparable for the CC and CT genotypes in both the YAZ Plus and YAZ. Mean plasma folate levels were lowest for the mutant (TT) genotype group ( $38.4 \pm 14.3$  nmol/L) in the YAZ Plus treatment group. The mean plasma folate level for YAZ Plus increased during the first 4 to 8 weeks after treatment initiation and reached steady state for the remainder of the study (Figure 2). For YAZ, the mean plasma folate level of the available (CC and CT) genotypes remained comparable to baseline values throughout the treatment period. For the YAZ Plus group, the mean change from Baseline was similar for all 3 genotypes at all time points. For the TT genotype, a mean change from Baseline of  $17.2 \pm 16.0$  nmol/L was observed for Week 24 compared with  $14.6 \pm 21.4$  and  $16.7 \pm 20.5$  nmol/L for the CT and CC genotypes, respectively.

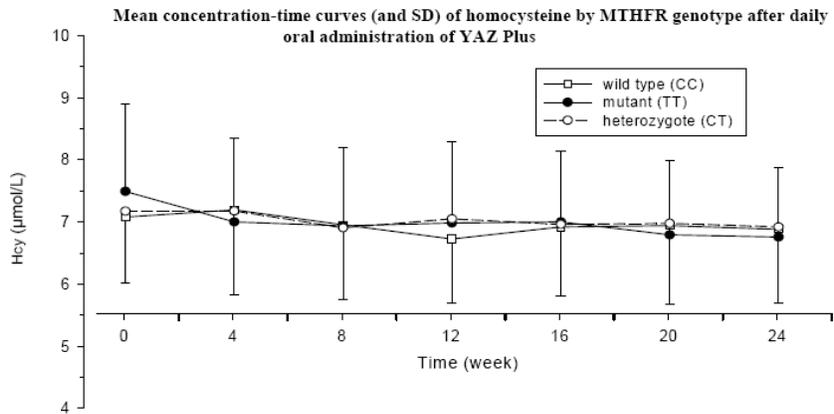
Figure 2:



Baseline mean  $\pm$  SD homocysteine levels were comparable for the CC and CT genotypes in both the YAZ Plus and YAZ treatment groups. Mean homocysteine levels were highest for the mutant (TT) genotype group in the YAZ Plus treatment group. The mean homocysteine level decreased slightly during the first 4 to 12 weeks after treatment initiation and then reached a steady state concentration for the remainder of the study (Figure 3). For YAZ, the mean homocysteine levels of the available (CC and CT) genotypes remained comparable to Baseline values throughout the treatment period. For the YAZ Plus group, the mean change from Baseline was highest for the

TT genotype group. For the TT genotype a mean change from Baseline of  $-0.7 \pm 1.0 \mu\text{g/L}$  was observed for Week 24, compared with  $-0.3 \pm 0.9$  and  $-0.2 \pm 0.9 \mu\text{g/L}$  for the CT and CC genotypes, respectively.

Figure 3:



Therefore, mean RBC and plasma folate levels at Baseline were comparable for the wild type (CC) and heterozygote (CT) genotype groups and lowest for the mutant (TT) genotype group. For the homozygote (mutant) TT genotype group of MTHFR polymorphism 677C>T, an increase in plasma and red blood cells (RBCs) was observed in the YAZ Plus group that was similar to the CC (wild type) and the CT (heterozygote) genotype groups. For homocysteine, baseline level was slightly higher in TT (mutant) group compared with the other genotype groups. During treatment, homocysteine levels are similar for all genotypes. Sponsor did not provide results from other genetic polymorphisms.

#### 4. COMMENTS

1. The sponsor did not provide the agency with patient level data or data on the other genetic variants (MTHFR 1415A>C, MTHFD1 1958 G>A, SLC19A1 80 A>C and DHFR IVS1+59) citing privacy reasons and the exploratory nature of the analyses. This greatly limits the interpretability of the analysis.

2. With the caveat that primary FDA analysis was not performed, there does not appear to be significant relationship between the MTHFR 677 C>T variant and folate responses to Yaz or Yaz Plus.

#### 5. RECOMMENDATIONS

The Office of Clinical Pharmacology/Genomics Group has reviewed the information contained in NDA 22-532. The sponsor's analysis will not impact approvability or labeling of the product submitted under this NDA.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22532	ORIG-1	BAYER HEALTHCARE PHARMACEUTICA LS INC	YAZ Folate
NDA-22532	GI-1	BAYER HEALTHCARE PHARMACEUTICA LS INC	YAZ Folate

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/s/

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